# Potential antimycobacterial agents targeting dihydrofolate reductase

El-Hamamsy, Mervat Hamed Rabu Ibrahem

*Award date:*
2005

*Awarding institution:*
University of Bath

# Potential Antimycobacterial Agents Targeting Dihydrofolate Reductase

Mervat Hamed Rabu Ibrahem El-Hamamsy

A thesis submitted for the degree of Doctor of Philosophy (PhD)

University of Bath

Department of Pharmacy and Pharmacology

July 2005

## COPYRIGHT





ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

## Abstract

Tuberculosis (TB) conjures up the image of a contagious, chronic, severe disease of the lungs that is often fatal without treatment. TB is caused by *Mycobacterium tuberculosis*, a slow-growing, acid-fast obligate aerobe that invades host macrophages and is transmitted mainly by inhalation.

TB can take two forms: primary TB, usually a mild disease; and secondary TB, a disease caused by reactivation of dormant organisms. TB is currently treated with a combination of antibiotics for at least six months to prevent the emergence of multidrug-resistance tuberculosis.

Dihydrofolate reductase (DHFR) is an enzyme that present in all cells and is required for normal folate metabolism in prokaryotes and eukaryotes. DHFR is a key enzyme in the biosynthesis of RNA, DNA and proteins. The three dimensional structure of Mtb-DHFR revealed the presence of a glycerol pocket in the active site which was absent in the human DHFR. We have studied the glycerol pocket as a new avenue for designing *novel* selective inhibitors to Mtb-DHFR.

Four groups of inhibitors were designed having 2,4-diaminopyrimidine nucleus with four different new functional groups at 6-position. We describe the synthetic pathway of the designed compounds which started by the condensation of phenylacetonitrile derivatives with esters or lactones to form the corresponding β-ketonitriles which, in turn, were O-methylated with diazomethane to form the corresponding enol ethers. Enol ethers were cyclised with guanidine to form the target 2,4-diaminopyrimidine derivatives.

Evaluation of the synthesised DHFR inhibitors was performed by initial screening in two types of genetically engineered strains of the budding yeast that are dependent on the Mtb-DHFR and human DHFR respectively for their growth. As expected the new functional group at 6-position provided selectivity as well as potency to the synthesised compounds as three *novel* inhibitors was more potent and more selective as inhibitors to Mtb-DHFR compared with trimethoprim.

# Contents

## Abbreviations used

| | |
|---|---|
| AIDS | Acquired immune deficiency syndrome |
| Ala | Alanine |
| aq. | Aqueous |
| Asp | Aspartic acid |
| BCG | Bacilli Calmette-Guerin vaccine |
| Bn | Benzyl |
| br | Broad |
| Brine | Saturated aqueous NaCl |
| c | Concentration |
| *C. albicans* | *Candida albicans* |
| CNS | Central nervous system |
| COSY | Correlation spectroscopy |
| CS | Cycloserine |
| d | Doublet |
| dd | Doublet of doublets |
| ddd | Doublet of doublet of doublets |
| DDMP | Metoprine |
| DHF | 7,8-Dihydrofolate |
| DHFR | Dihydrofolate Reductase |
| DME | 1,2-Dimethoxyethane |
| DMF | Dimethylformamide |
| DMSO | Dimethylsulfoxide |
| DMSO-$d_6$ | Deuterated dimethylsulfoxide |
| dt | Doublet of triplets |
| DNA | Deoxyribonucleic acid |
| DOTS | Directly observed treatment, short-course |
| dTMP | Deoxythymidylate monophosphate |
| dUMP | Deoxyuridine monophosphate |
| *E. coli* | *Escherichia coli* |
| EMB | Ethambutol |
| FAB+ve | Fast atom bombardment, positive ion |
| FAB-ve | Fast atom bombardment, negative ion |

| | |
|---|---|
| $FH_4$ | 5,6,7,8-Tetrahydrofolate |
| Gln | Glutamine |
| Glu | Glutamic acid |
| Gp-170 | Membrane-bound P-glycoprotein |
| h | Hour |
| H-bond | Hydrogen bond |
| h-DHFR | Human dihydrofolate reductase |
| HIV | Human immunodeficiency virus |
| Hu | Human |
| INH | Isoniazid |
| IR | Infra-red |
| *J* | Coupling constant |
| LAM | Lipoarabinomannan |
| LDA | Lithium diisopropylamide |
| Leu | Leucine |
| L-J | Lowenstein-Jensen growth medium which is composed of whole eggs, glycerol, other nutrients and trace elements |
| LSA | Lithium bis(trimethylsilyl)amide |
| m | Multiplet |
| *M* | Mycobacterium |
| MBP | Methylbenzoprim |
| MDR | Multi-drug resistance |
| Me | Methyl |
| min | Minutes |
| MNNG | N-methyl-N-nitro-N-nitrosoguanidine |
| mp | Melting point |
| MS | Mass spectrometry |
| Mtb | *M. tuberculosis* |
| NADPH | Dihydronicotinamide adenine dinucleotide phosphate |
| NBA | 3-Nitrobenzyl alcohol |

| | |
|---|---|
| NMR | Nuclear magnetic resonance |
| NMU | N-methyl-N-nitrosourea |
| PAZ | Pyrazinamide |
| *P. carinii* | *Pneumocystis carinii* |
| PCC | Pyridinium chlorochromate |
| PDB | Protein Data Bank |
| Pd/C | Palladium on activated charcoal |
| Pet. ether | Petroleum ether |
| Pf | *Plasmodium falciparum* |
| Ph | Phenyl |
| Phe | Phenylalanine |
| POA | Pyrazonic acid |
| Pro | Proline |
| PTX | Piritrexim |
| ppm | Part per million |
| q | Quartet |
| qn | Quintet |
| QSAR | Quantitative structure activity relationship |
| RMP | Rifampin |
| RNA | Ribonucleic acid |
| ROI | Reactive oxygen intermediates |
| s | Singlet |
| $S_N2$ | Bimolecular nucleophilic substitution |
| $S_NAr$ | Aromatic nucleophilic substitution |
| SOD | Superoxide dismutase |
| STM | Streptomycin |
| t | Triplet |
| TB | Tuberculosis |
| TBDMS | *tert*-butyldimethylsilyl |
| TBDPS | *tert*-butyldiphenylsilyl |
| TCH | Thiophene-2-carboxylic acid hydrazide |
| tert | Tertiary |

| | |
|---|---|
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| TMP | Trimethoprim |
| TMQ | Trimetrexate |
| TMS | Tetramethylsilane |
| TMX | Methotrexate |
| Trp | Tryptophan |
| UV | Ultra-violet |
| WHO | World Health Organization |

## List of Figures, Schemes and Tables

## Schemes

## Tables

## Acknowledgments

I would like to thank my supervisors Prof. A. W. Smith, Dr. A. S. Thompson and Dr. M. D. Threadgill for their unlimited support and help throughout my research project.

Thanks to Dr. S. Black and Mr. D. Wood for the provision of NMR spectra and to Mr. C. Cryer for the mass spectra data. I also would like to thank Mr. K. Smith for help in using the Midas Plus program and Ms. J. Carter for help in the microbiological experiments.

I would like to thank my colleagues in the research group Christopher, Claire, Ghadeer, Esther, Christian, Joey, Anna-Marie, Dr. Victoria, Archana and Sabela.

I would like also to thank all the technical, research and administrative staff from the Department of Pharmacy and Pharmacology for their help during this project.

I acknowledge and appreciate the financial support of the Government of the Arab Republic of Egypt.

Finally, I would like to express my greatest thanks to my family and friends for their help and encouragement.

My special thanks go to my husband Alaa and my children Mohamed, Nora and Nada for their endless support and help.

# 1. INTRODUCTION

## 1.1 The Genus *Mycobacterium*

The generic name *Mycobacterium* was introduced by Lehmann and Neumann in the first edition of their "Atlas of Bacteriology" published in 1896.[1] Etymologically, "*Mycobacterium*" is derived from the Greek for fungus (myces) and small rod (bacterion). The fungus component of the name derives from the tendency of these microorganisms to spread diffusely over the surface of liquid medium in a mould-like growth pattern.[2] The minimal standards for identifying bacilli as members of the genus *Mycobacterium* are as follows:

1. Resistance to weak acid decolourisation following staining by carbolfuchsin (basic dye) and similar dyes.
2. The unique structure of the cell wall envelope due to the presence of mycolic acids containing 60-90 carbons, which can be cleaved to 22-26 carbon fatty acid methyl esters by pyrolysis.
3. A guanine and cytosine DNA content of 61-71 mol%.[3]

### 1.1.1 The Species of *Mycobacteria*

Historically, the most widely used system for classification of mycobacterium was that devised by Adanson, in which a large number of cultural and biochemical properties were used to group strains into clusters according to their similarity. This technique was applied successfully to mycobacteria by The International Working Group on Mycobacterial Taxonomy.[1] Mycobacteria are divisible into the rapid growers, the slow growers and those not yet cultivated *in vitro*. The clinical microbiologist finds it useful to divide them into the major pathogens of human and animals, those species that frequently cause opportunist infections, and those that rarely or never do so, table (1).[1,3] A general, but not exclusive, trend regarding mycobacterial species is that the more "slow-growing" species contain most of the highly pathogenic organisms, including *M. tuberculosis*, *M. bovis* and *M. leprae*. In contrast the "rapid growers," with the exception of a few opportunistic species such as *M. chelonae* and *M. fortuitum*, tend to be nonpathogenic. Still, as Shinnick and

Good have pointed out, there may be no such thing as a nonpathogenic mycobacterium in the severely immunocompromised host.[2,3]

| Species | Clinical disease |
|---|---|
| **Slow growers** | |
| *M. tuberculosis* | Tuberculosis |
| *M. bovis* | Bovine tuberculosis |
| *M. avium* | Disseminated infection in AIDS patients |
| *M. intracellulare* | |
| *M. kansasii* | Lung infections |
| *M. marinum* | Skin infections and deeper infections (*e.g.* arthritis) associated with aquatic activity |
| *M. scrofulaceum* | Cervical adenitis in children |
| *M. simiae* | Lung, bone and kidney infections |
| *M. szulgai* | Lung, skin and bone infection |
| *M. ulceranes* | Skin infections |
| *M. xenopi* | Lung infections |
| *M. paratuberculosis* | Associated with Crohn's disease |
| **Rapid growers** | |
| *M. fortuitum* | Opportunist infections with introduction of organisms into deep subcutaneous tissues; usually associated with trauma or invasive procedures |
| *M. chelonea* | |
| **Non-cultivable** | |
| *M. leprae* | leprosy |
| Slow growers require more than 7 days for visible growth from a dilute inoculum; rapid growers require less than 7 days for visible growth from dilute inoculum | |

**Table (1) Major species of *Mycobacterium* associated with human disease**

At the present time, the emphasis is on the direct study of the genome, for example, by the sequence analysis of the DNA coding for the 16S ribosomal RNA. This has minor variations in its base sequence that appear to correspond closely with

the established mycobacterial species. Thus determination of the sequence of the bases in this RNA may be used to classify mycobacteria and to identify clinical isolates.[4]

### 1.1.2 The Structure of the Mycobacterial Cell

The mycobacterial cell consists of cytoplasm bounded by a plasma membrane and enclosed by a complex lipid-rich cell wall. The single chromosome is tightly wrapped into a nuclear body but is not bounded by a nuclear membrane. Thus, like other bacteria, the mycobacteria are prokaryotes (higher unicellular and multicellular forms of life have nuclear membranes and are termed eukaryotes). Some mycobacteria contain additional small circles of DNA, termed plasmids. The cell membrane consists of a bilayer of polar phospholipids with their hydrophobic ends facing inwards and their hydrophilic ends facing outwards. The membrane is closely associated with the enzymes and cofactors involved in energy production.[5]

### 1.1.3 Morphology and Staining

Mycobacteria are complex unicellular organisms. They are thin, slightly curved to straight, non-spore-forming, non-motile bacilli, measuring 0.2 to 6.0 μm × 1.0 to 10 μm. The genus consists of more than 50 species.[6] They are gram-positive but many species stain poorly with this stain even after prolonged staining, because of the large content of lipid within the cell wall. A distinguishing characteristic of mycobacteria species relates to their ability to retain a primary carbofuchsin stain after treatment of organisms with an acid alcohol wash. This characteristic is referred to as acid fastness.[7]

### 1.1.4 Sensitivity to Physical and Chemical Agents

Although mycobacteria can survive for several weeks in the dark, especially under moist conditions, and for many days in dried sputum on clothing and in dust, they are rapidly killed by ultraviolet light even through glass, and by heat (60° C for 15-20 min, or by autoclaving). They are susceptible to phenol and phenolic derivatives and to glutaraldehyde, provided that the contact time is adequate. Phenols

such as Hycolin are useful at 5% final concentration in discard flasks for supernatant fluids. The most useful disinfectant is 70% alcohol. This is efficient and clean and leaves the surface free of any deposit.[8,9]

### 1.1.5 Drug Sensitivity

With the exception of the rapid growers, most mycobacteria are fairly resistant to conventional antimicrobials. Accordingly, specific antimicrobial drugs and treatment regimens have been developed. Until 50 years ago, there were no drugs to cure TB.[10]

## 1.2 *Mycobacterium Tuberculosis*

*M. tuberculosis* is the causative organism of tuberculosis (TB) in humans. The tubercle bacillus, because of its unusual wax envelope, grows slowly and is highly successful parasite; it does not affect the life of its victim for many years. When it causes extensive damage to the lungs, it ensures its spread from the body into the environment and increases its chances of infecting other people.

Mycobacteria grow very slowly; the generation time of tubercle bacilli is about 13 hours on the best laboratory media such as Lowenstein-Jensen (L-J) medium which is composed of whole eggs, glycerol, other nutrients and trace elements. It is possible that slow growth results from inability to transport nutrients rapidly across the wax layer. Slow growth causes delay in the diagnosis by culture; laboratory cultures of clinical material must be incubated for up to 8 weeks. It also greatly delays testing the drug sensitivity of clinical isolates; this delay has tragic consequences for AIDS patients infected with drug-resistant tubercle bacilli. The patient may die without receiving the most effective drugs.[11]

*M. tuberculosis* is divisible into two major types: the classical type and the South Indian or Asian type. The latter type was originally isolated in the Chennai area of the South India and differs from the classical type in being attenuated in guinea-pig and being susceptible to killing by hydrogen peroxide *in vitro*. This variant was subsequently found to be susceptible to the isoniazid analogue

thiophene-2-carboxylic acid hydrazide (TCH). About 60% of tubercle bacilli isolated in the Chennai region are of this type and it is also found in Asian communities who have settled in other parts of the world. Despite its low virulence to guinea pig, it appears fully virulent for humans and it causes disease similar to the classical strains.[12]

### 1.2.1 Tuberculosis Overview: The Current Situation

TB is a major killer disease and responsible for a higher number of deaths than any other single infectious disease.[11] In 1993, the World Health Organization (WHO) declared TB a "global health emergency", drawing attention to a problem that had been largely ignored over the previous few decades. Rates of TB continue to rise in developing and industrialised nations alike, leading to an estimated eight million new cases every year and a death toll of three million. Several factors have contributed to this increase, such as movement of people around the world, homelessness in major cities and, perhaps of greatest significance, the HIV pandemic.[13] TB is primarily a disease of the lung but may spread to other sites or proceed to a generalised infection.[14]

HIV and TB form a lethal combination, each speeding the other's progress. TB is a leading cause of death among people who are HIV-positive. It accounts for about 13% of AIDS deaths worldwide. In Africa, HIV is the single most important factor determining the increased incidence of TB in the past 10 years.[15]

### 1.2.2 The Present Global Burden of Tuberculosis

Nearly one third of the global population (that is, 2 billion people) is infected with TB or is at risk of developing the disease.[9] WHO estimated that there were 8.8 million new cases of TB in 2002, of which 3.9 million developed active TB and 2 million died. The global incidence rate of TB is growing at approximately 1.1% per year and the number of new cases at 2.4% per year.[10,16] TB accounts for 2.5% of the global burden of disease and for 26% of preventable deaths and is the commonest cause of death in young women. Some 95% of global TB cases and 98% of deaths occur in the developing world, where 75% of cases are in the economically most

productive age group (15-54 years). There, on average, three to four months of work time are lost if an adult has TB. This results in the loss of 20-30% of annual household income and an average of 15 years' income if the patient dies.[13,15]

In addition to the devastating economic cost, TB imposes indirect negative consequences. In India alone every year, more than 300,000 children leave school because of their parents' TB and more than 100,000 women are abandoned by their families because of their TB. TB impoverishes and poverty attracts TB. Furthermore, co-infection with HIV significantly increases the risk of developing TB. Today, worldwide, 11 million people are co-infected with TB and HIV. The TB/HIV co-epidemic is increasing and will continue to fuel the TB epidemic. Of the 3.6 million cases of TB cases reported to the WHO, South-East Asia and the Western pacific regions accounted for almost 60% of the global caseload, with India and China alone reporting more than 40% of all notified cases (fig. 1).[15]

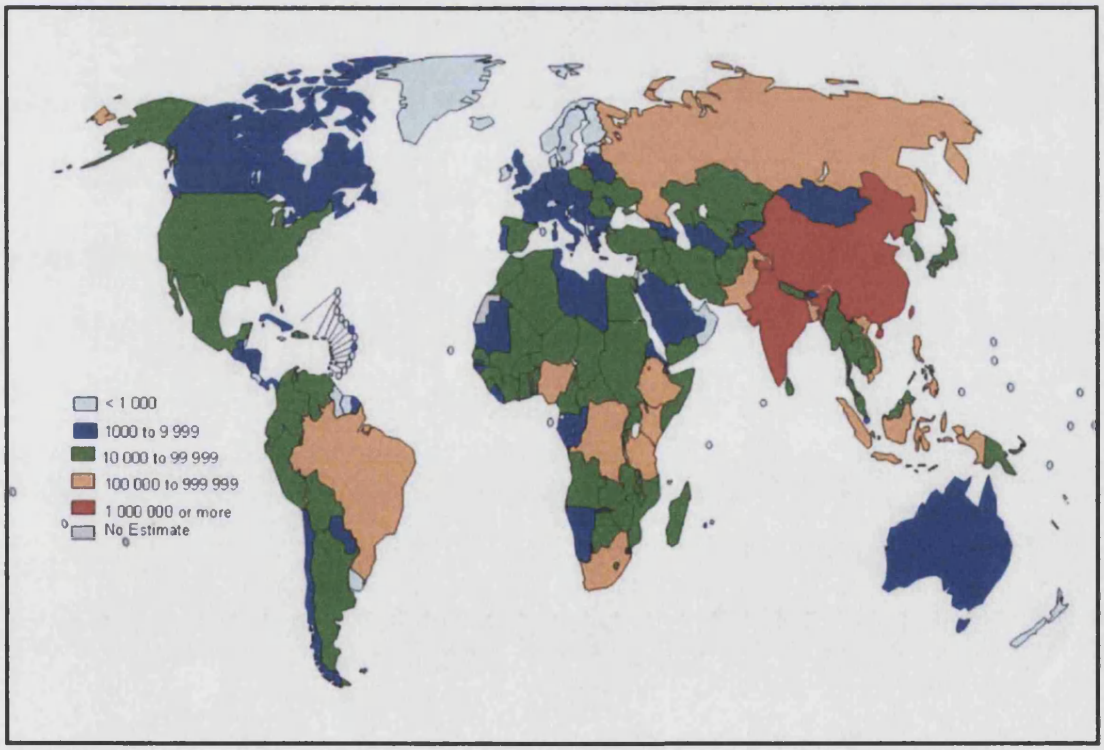



**Fig. (1) Global distribution of tuberculosis, World Health Organization (2002)**

Although Africa contains only 10% of the 5.9 billion global populations, the region reported 18% of the total TB cases where HIV has led to rapid increases in the incidence of TB and increases the likelihood of dying from TB (fig. 2,3).[15,17]

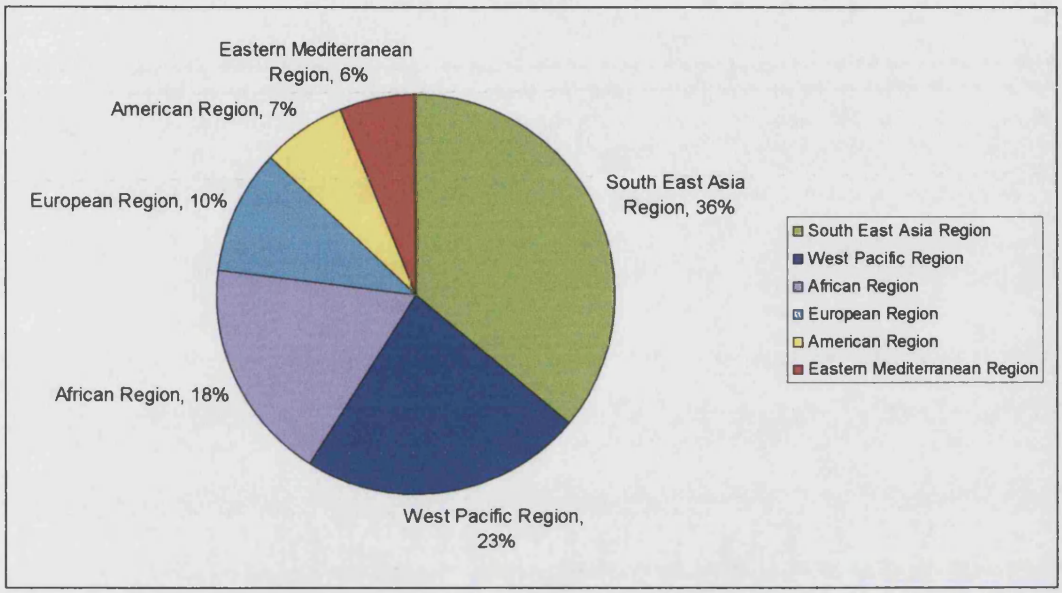



**Fig. (2) Tuberculosis case notification proportion by WHO regions**

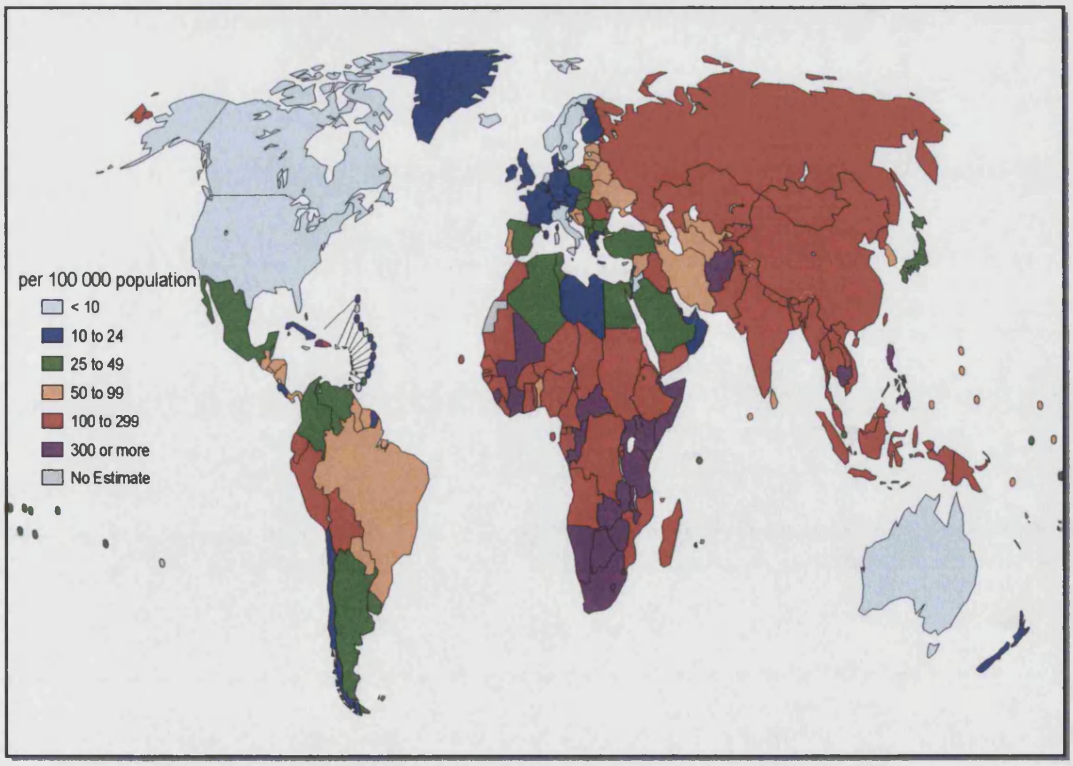



**Fig. (3) The global rate of incidence of TB, WHO (2002)**

### 1.2.3 The Terminology and Classification of Tuberculosis

TB is divided into two main types: primary and post-primary (secondary). In the past, it was widely assumed that post-primary disease was always due to endogenous reactivation of an earlier primary infection, usually after a period of latency. It is now realised that this type of TB may be also the result of exogenous reinfection. TB is also divided according to whether or not the lung is involved. The terms non-pulmonary and extrapulmonary are often used synonymously but it is recommended that the former should be used to refer to disease in which the initial infection was not in the lung and the latter to lesions due to spread from an initial pulmonary focus. The clinical manifestations of TB are so variable in type, extent and site that any classification, other than by the simple categorisation above, is of little value.[18]

### 1.2.4 Aetiology and Transmission

The causative agent of TB is almost always *M. tuberculosis* with rare instances of three other species of the Mtb complex namely, *M. bovis*, *M. africanum* and *M. microti*.[14] Infection is acquired by inhalation of *M. tuberculosis* in aerosols and dust.[10] TB is almost universally transmitted through the air by infectious particles called droplet nuclei.[14,19] When a person with active pulmonary or laryngeal TB coughs (0 to 3,500 particles), sneezes (4,500 to 1,000,000 particles) or even speaks (0 to 210 particles), these particles are emitted in droplets that can remain suspended in air for several hours.[18]

Transmission may occur if another person inhales the droplet nuclei, as the bacilli will be deposited in the lungs of contacts resulting in local infection, then dissemination.[14,19] Less frequently, bacilli may be ingested and lodge in the tonsil or in the wall of the intestine. Such infection is particularly associated with the consumption of contaminated milk or milk products. A third, but rare, mode of infection is direct implantation of bacilli into the skin through cuts and abrasions. This is a health hazard faced by those working with infected material or cultures of tubercle bacilli.[18]

Three factors determine the probability that TB will be transmitted; the infectiousness of the person with TB, the environment in which the exposure occurred and the duration of the exposure.[14,18] Airborne transmission of TB is efficient because infected people cough up enormous numbers of mycobacteria (1-10 million bacilli per milliliter of sputum), projecting them into the environment, where their waxy outer coat allows them to withstand drying and thus to survive for long periods of time in air and house dust.[14]

### 1.2.5 Pathogenesis

Tubercle bacilli do not produce exotoxins or endotoxin. The severe manifestations of TB are largely mediated by the defensive responses that the host's immune systems mount against the bacilli.[18] The upper respiratory tract is the body's first line of defence against the transmission of TB. Particles smaller than five microns in diameter may reach the alveoli and infection may begin.[20]

The pathogenicity of the tubercle bacillus arises from strategies it has developed to survive in host cells, including the ability to colonise inside the macrophages, and to remain quiescent and then become active decades later. The presence of storage proteins in the bacillus points to its ability to stockpile essential growth factors, allowing it to persist in the nutrient-limited environment. In this regard, the ferritin-like proteins may be important in intracellular survival, as the capacity to acquire enough iron in the vacuole is very limited.[20,21]

### 1.2.6 Primary Tuberculosis

This is the disease of persons who are infected for the first time. The organisms are phagocytosed by the alveolar macrophages where they can both survive and multiply. Bacilli multiply in this intracellular environment until the macrophages burst and release them.[20] Later, non-resident macrophages are attracted to the site and these also ingest the mycobacteria and carry them *via* the lymphatic system to the local (hilar) lymph nodes, where an immune response develops, dominated by T-helper cells. Inflammation will now be present in several places: at the original site of infection, along lymphatic channels, and in the regional lymph

nodes. The development of inflammation takes (4-10) weeks. The immune defences curb the proliferation of the organisms and retard their local spread through phagocytosis, although some may survive phagocytosis, grow intracellulary, rupturing the macrophages and give rise to haematogenous spread to distant organs.[14] The process of phagocytosis is shown in (fig. 4).[22]

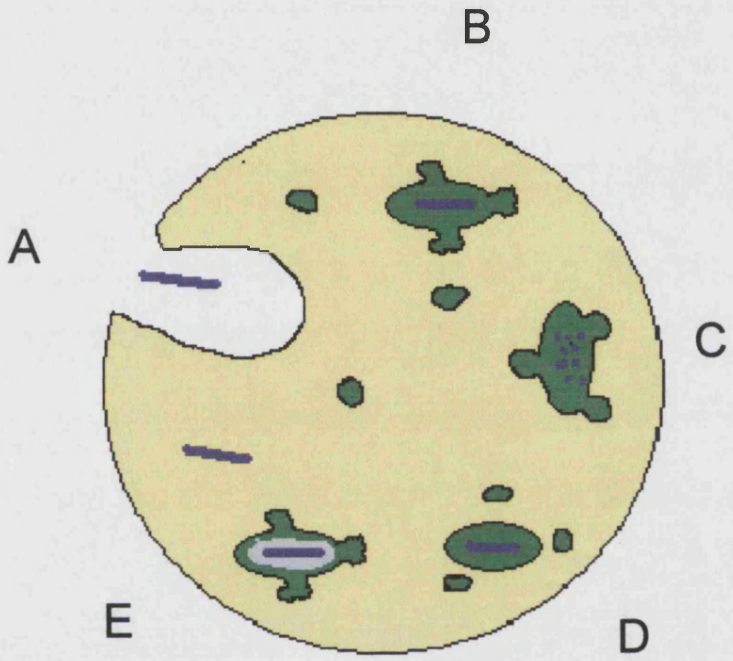



**Fig. (4) Phagocytosis, A**: Bacilli are engulfed by the cell membrane, **B**: The bacilli lie in a membrane vesicle (the phagosome) which fuses with lysosomes containing bactericidal substances, **C**: These substances destroy the bacilli. Mycobacterium avoids such destruction by – **D**: inhibiting phagosome/lysosome fusion; **E**: a thick capsule-like outer protective layer; and escape from the phagosome and lie freely within the cytoplasm.

The phagocytosis occurs once an engulfed bacterium by a macrophage lies within a vesicle formed by invagination of the surface membrane. This vesicle, the phagosome, then fuses with the lysosomes which contain bactericidal agents. There are three main strategies by which mycobacteria survive within the phagosome. Firstly, phagosome/lysosome fusion may be inhibited. Secondly, the pathogen may

cover itself with a protective layer that absorbs or neutralises the bactericidal agents or, thirdly, it may escape from the vesicle and lie freely in the cytoplasm of the cell.[20,22]

The extent to which mycobacteria use these strategies is controversial. *Mycobacterium tuberculosis*, in common with the protozoal parasite *Toxoplasma gondii*, inhibits phagosome-lysosome fusion but neither the mechanism nor the significance of this activity to intracelluluar survival is clearly understood. Mycobacterial pathogens also appear to survive the effects of exposure to reactive oxygen intermediates (ROI) on account of the thick outer layer of mycosides which, on electron microscopy, appears as an electron transparent zone surrounding the bacilli. Lipoarabinomannan (LAM), which is present in all mycobacteria, also protects against ROI. In addition, mycobacteria secrete the enzyme superoxide dismutase (SOD) which also protects against ROI.

Mycobacteria are able to escape from the phagosome and replicate in the cytoplasm. This appears to occur when the cell becomes immunologically effete and unable to control intracellular growth or when mycobacteria enter cells other than macrophages.[22] Sensitised T-cells release lymphokines that activate macrophages and increase their ability to destroy the mycobacteria. The body reacts to contain the organisms within tubercles, small granulomas consisting of epithelioid cells, giant cells and lymphocytes. The lung lesion plus the enlarged lymph nodes is often called the Ghon (or primary) complex.[20]

Within time, the centres of the tubercles become necrotic and advance to form cellular masses of cheesy debris, called caseous material or caseation. Primary TB may take two courses: In people who are otherwise healthy, the lesions heal spontaneously and become fibrotic or calcified. These lesions usually persist as such for a life-time. In contrast to healthy individuals, in an immunocompromised person, the mycobacteria are not contained within the tubercles but invade the blood stream and cause disseminated disease. The involvement of macrophages has its price. Two cytokines produced by these cells, interleukin-1 which acts as mediator of the fever experienced by tuberculosis patients, and tumour necrosis factor, which interferes with lipid metabolism and leads to severe weight loss.[14,20]

### 1.2.7 Secondary (post-primary) Tuberculosis

Secondary TB usually becomes noticeable one or two years or even decades after primary disease.[14] Secondary TB is due to reactivation of dormant mycobacteria, usually as a consequence of impaired immune function resulting from some other cause such as malnutrition, infection (*e.g.* AIDS), cancer chemotherapy or corticosteroids for treatment of inflammatory diseases. Reinfection with external Mtb could lead to the same manifestation.

Reactivation occurs most commonly in the apex of the lungs. This site is more highly oxygenated and has impaired lymphatic drainage which allows the mycobacteria to multiply more rapidly to produce caseous necrotic lesions, which, in time, liquefy and discharge their contents into bronchi.[20,23] The softened contents are coughed out, leaving a well-oxygenated cavity in which the organisms actively proliferate. Thus the cavity wall contains millions of freely replicating bacilli, which are behaving more like saprophytes than primary pathogens. In addition, large numbers of bacilli enter the sputum, rendering the patient open or infectious.[22] The discharge of caseous material also distributes the organisms to other sites in the lung, which can lead to rabidly progressive tuberculosis pneumonia. Coughing and spitting transmit the organisms to other people.[24] Inflammation of the surface of the bronchi causes increased mucus secretion, stimulation of the cough reflex; patients cough up sputum and destruction of tissues results in bloody sputum.[10,14]

### 1.2.8 Clinical Features and Diagnosis of TB

TB is a chronic bacterial infection of the lungs. The onset of TB is insidious, the infection proceeding for some time before the patient becomes sufficiently ill to seek medical attention. Primary TB is usually mild and asymptomatic and in 90% of cases does not proceed further. However, clinical disease develops in the remaining 10%.[10,20] Secondary infections may occur in any organ as the mycobacteria have the ability to colonise almost any site in the body. The clinical manifestations are variable; fatigue, weight loss, night sweats, chills, anorexia, weakness and fever are all associated with TB. Infection in the lungs characteristically causes chest pain, a chronic productive cough, and the sputum may be blood-stained as a result of tissue

destruction. Necrosis may erode blood vessels, which can rupture and cause death through haemorrhage. Approximately 15% of TB cases are extrapulmonary and the specific symptoms depend on the site of involvement. The most prevalent local symptom is pain at the affected site and the systemic symptoms are fever, malaise, anorexia, loss of weight and sweating. The local symptoms include cough, sputum and dyspnea.[10,14]

Generally, TB lymphadenitis is the most prevalent form of extrapulmonary TB, due to infection of the lymph nodes, seen in Great Britain and is common in patients of Asian ethnic origin. It is also, for unknown reasons, reported to be more prevalent in females in all ethnic groups. The affected nodes are initially discrete, rubber-like in texture and usually painless. Less than one half of the patients have constitutional symptoms.

Miliary TB , which is a type of disseminated TB, is more prevalent in males. It usually occurs as a manifestation of primary TB and is characterised by multiple discrete granulomas macroscopically resembling millet seeds. These occur throughout the body and, on chest X-ray, may produce a characteristic "snow storm" appearance. Occasionally they are seen on the retina by ophthalmoscope. Lesions also occur in the kidney and tubercle bacilli are found in the urine of about 25% of the patients with miliary disease.[25,26]

For a person who is suspected of having TB, a medical evaluation should include a medical history, a physical examination, a chest radiograph, appropriate bacteriological or histological examination and a tuberculin test. A positive tuberculine reaction usually manifests as an area of induration which reaches a maximum after 48 or 72 hours of intradermal injection of tuberculoprotein.[22]

When extrapulmonary TB is suspected, a variety of clinical specimens other than sputum (*e.g.* urine, cerebrospinal fluid, pleural fluid, and pus or biopsy specimens) may be needed for examination.[10,14] These materials are cultured to obtain confirmation that the acid-fast organisms are indeed *M. tuberculosis* and to obtain information on the sensitivity of the organism to different anti-tuberculous drugs. This process takes two to three months to provide results. A number of

factors, in particular HIV infection, multi-drug resistance (MDR) and rapid air transport of people around the world, causes difficulties with diagnostic approach which rendering it too slow and unreliable.

These concerns have lead to an urgent search for new techniques to assist in making the diagnosis of TB. The *M. tuberculosis* genome contains distinctive sequences which can be utilized to identify the organism. Many studies have taken advantage of this opportunity.[22,26] Thus, following culture, it is possible to extract DNA, digest it with enzymes and use specific probes for different mycobacteria to confirm which cultures are indeed *M. tuberculosis*, as opposed to other mycobacteria. There are now commercial kits, such as the Accuprobe system, which can identify a culture as belonging to *M. tuberculosis* complex in under two hours. This approach is widely used combined with the BACTEC system of rapid identification by liquid culture to provide results within two weeks.[27]

### 1.1.9 Treatment of TB

Most patients with TB can be cured with adherence to chemotherapeutic regimens. The goal of drug therapy of active TB is two fold; to cure the sick and to impede the transmission of tubercle bacilli in the community. On the basis of controlled clinical trials, three basic principles for the treatment of TB have evolved: (a) regimens for treatments of disease must contain multiple drugs to which the organisms are susceptible, (b) the drugs must be taken regularly, and (c) drug therapy must continue for sufficient period of time.

The aim of therapy is to provide the most efficacious regimen with the least toxicity possible for the shortest period of time.[28] Three subpopulations of tubercle bacilli can potentially coexist during an infection. Antituberculous agents are targeted toward various sites of mycobacterial growth in the body. As the first phase, tubercle bacilli in the lesions of patients with pulmonary TB lie tightly packed in very large number in a narrow zone close to the air-caseum interface. Since caseum is composed of dead cells, the bacilli are usually some distance from living macrophages and are extracellular.[29] The most numerous population consists of the extracellular bacteria; these organisms are killed most readily by isoniazid (INH) and

streptomycin (STM) and to a lesser extent by rifampin (RMP). The second population is composed of organisms that seek out the acidic environment of caseating granulomas. RMP exhibits the greatest activity in killing these organisms. The final population of organisms exists within the activated macrophages (intracellular). Pyrazinamide (PAZ) possesses the greatest activity against this population.[28,30]

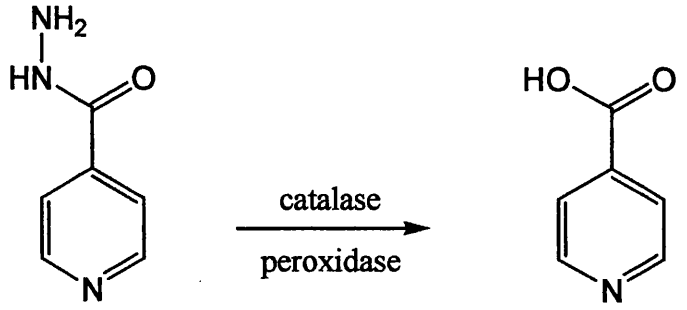
### 1.2.10 Current TB Drugs and Their Targets

Current TB drugs can be divided into first-line drugs; INH, RMP, PAZ, STM, ethambutol (EMB), and second-line drugs; *p*-aminosalicylic acid (PAS), cycloserine (CS), kanamycin, capreomycin, thiacetazone, fluoroquinolones. TB drugs can also be classified according to their specificity into TB-specific drugs (INH, PZA, EMB, PAS, CS, EMB, thiacetazone) and broad-spectrum drugs (RMP, STM, kanamycin, amikacin, capreomycin, fluoroquinolones).[30] The first-line drugs, also known as primary or standard drugs, are those recommended for the treatment of newly diagnosed TB. The other antituberculosis medications are considered second line, secondary, or reserve drugs, as they are used in case of resistance or intolerance to the otherwise preferred first-line drugs.[28]

### 1.2.11 The Mechanism of Action of the First-Line Antituberculosis Drugs

#### I) Isoniazid (INH)

Since its introduction in 1952, INH has been the most widely used antituberculous agent. The drug exhibits many qualities of an ideal agent: it is bactericidal, relatively nontoxic, inexpensive and well-absorbed orally or parenterally.[14] INH is a prodrug that requires activation by the bacterial catalase-peroxidase (Kat G) to generate a range of reactive oxygen species and reactive organic derivatives, isonicotinic acid being the major product (scheme 1), which then attack multiple targets in the tubercle bacillus.[30,31]

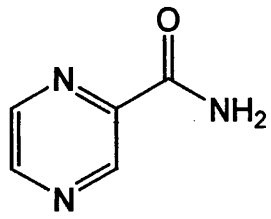



**Isoniazid (INH)** **Isonicotinic acid**

**Scheme (1) Isoniazid (INH) and its active metabolite isonicotinic acid**

The most well-known target is the cell wall mycolic acid synthesis pathway where at least two enzymes, Inh A (enoyl ACP(acyl carrier protein) reductase) and Kas A (β-ketoacyl ACP synthase), have been identified as targets of INH inhibition. INH activation generates various reactive organic and reactive oxygen radicals that could cause damage to a wide variety of cellular targets including DNA, carbohydrates and lipids. The cidal activity of INH is very likely to be due to its effect on multiple targets in tubercle bacillus.[30,32]

**II) Pyrazinamide (PZA)**




**Pyrazinamide (PZA)**

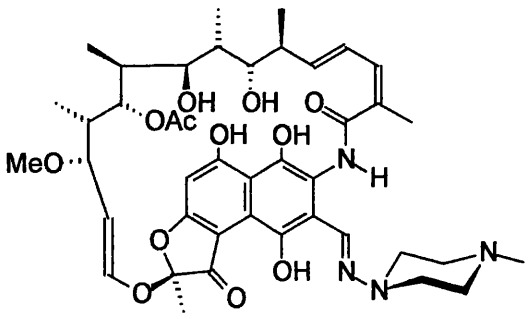
PZA, a synthetic pyrazine analogue of nicotinamide, exhibits bactericidal activity against mycobacteria in an acidic environment.[28] PZA is also a prodrug that requires activation or conversion to its active form, pyrazonic acid (POA), by pyrazinamidase/nicotinamidase enzyme.[33] PZA is a unique and paradoxical TB drug: it has remarkable *in vivo* activity and is involved in shortening the TB therapy, yet it has no activity against the TB bacteria at normal culture conditions except at acidic

pH.[30] The role of acidic pH is to facilitate the formation of uncharged protonated POA that can permeate the membrane easily; the acid-facilitated POA influx overwhelms the weak POA efflux mechanism and causes accumulation of POA in *M. tuberculosis*.[34]

The protonated POA brings protons into the cell and can eventually causes cytoplasmic acidification, which can inhibit vital enzymes. Protonated POA could also potentially de-energise the membrane by collapsing the proton motive force and affecting membrane transport. While the acid-facilitated uptake of the weak acid POA is a non-specific process, the specificity and unique susceptibility of *M. tuberculosis* to PZA is most likely to be due to a combination of deficient POA efflux mechanism and poor ability to maintain its membrane potential (as in starved and nongrowing bacterial populations).

This view is consistent with the observation that PZA/POA is more active against semi-dormant organism or old bacilli in stationary phase cultures where energy reserve and membrane potential is less well maintained than actively growing bacilli in the log phase. Thus the target of PZA/POA appears to be the membrane as they cause disruption of the membrane function and energy metabolism. A recent study using a related mycobacterium and a related compound, 5-chloro-PZA, suggested fatty acid synthase-1 as target for PZA, and this finding remains to be confirmed for *M. tuberculosis*.[30]

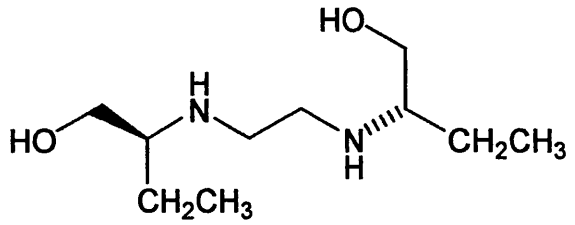
**III) Rifampin (RMP)**




**Rifampin (RMP)**

RMP is a semisynthetic derivative of rifamycin B, which is an antibiotic produced by *Streptomyces mediterranei*. RMP is an important agent in the treatment of TB, leprosy and diseases caused by the nontuberculous mycobacteria.[29] RMP interferes with RNA synthesis by binding to the bacterial DNA-dependent RNA polymerase β-subunit.[30,35] RMP is considered to be bactericidal and is active against both intracellular and extracellular *M. tuberculosis*. Its use in combination with INH in treatment regimens for TB allowed the duration of therapy to be decreased from 18-24 months to 6-9 months.[28]

**IV) Ethambutol (EMB)**

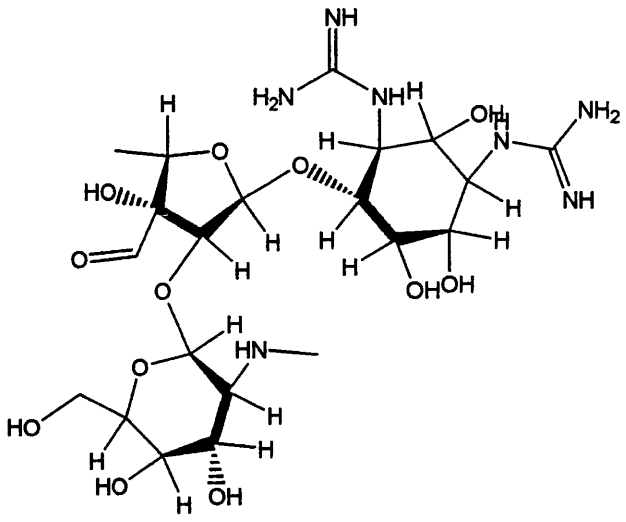



**Ethambutol (EMB)**

Ethambutol hydrochloride is a synthetic agent that was developed from N,N'-diisopropylethylenediamine, which was found to be active against *M. tuberculosis* in a screening program. The *d*-enantiomer of EMB is approximately 200 times more active than the *l*-enantiomer. [36,37]

EMB acts on the biosynthesis of arabinogalactan, a major polysaccharide of the mycobacterial cell wall. EMB inhibits the polymerisation of cell wall arabinan of arabinogalactan and of lipoarabinomannan and induces accumulation of β-D-arabinofuranosyl-*p*-decaprenol, an intermediate in arabinan bisynthesis.[34,38]

**V) Streptomycin (STM)**



**Streptomycin (STM)**

STM, an aminoglycoside antibiotic, was the first clinically effective drug to become available for the treatment of TB. STM is bactericidal in alkaline environments and acts by inhibiting protein synthesis.[30]

Because aminoglycosides are poorly absorbed from the gastrointestinal tract, STM must be administered parenterally. The drug is highly effective within the extracellular environment; however, it diffuses poorly into granulomas and macrophages and lacks activity in the intracellular environment.[28]

### 1.2.12 Current TB Therapy

Current antituberculous regimens use combinations to kill the slowly dividing organisms within granulomas and macrophages and minimise drug resistance.[15] Early in the chemotherapy era, it become apparent that the emergence of resistance during monotherapy could be circumvented with combination therapy.[14,28]

Current TB therapy consists of an initial phase of treatment with four drugs, INH, RMP, PZA and EMB for 2 months daily, followed by a continuation phase of treatment with INH and RMP for another 4 months, three times a week. This therapy,

also called DOTS (directly observed treatment, short-course), is the best TB therapy and is recommended by WHO for treating every TB patient. DOTS has a cure rate of up to 95%, given patient compliance.[38]

Despite the importance of DOTS in the control of TB, there are indications that in areas where there is high incidence of multidrug-resistant tuberculosis (MDR-TB), (which is the case of TB that exhibits resistance to the two most powerful antituberculous medications, INH and RMP) such as in Russian prisons and some African countries, DOTS is failing to control the disease.[39,40] In view of this, DOTS-Plus (DOTS plus second-line TB drugs) is now recommended for treating MDR-TB and TB in areas with high incidence of MDR-TB.[38] However, DOTS-Plus is expensive, takes longer to administer and has significant side-effects.[30]

### 1.2.13 Problems of the Current TB Therapy

Although DOTS is the best treatment for TB, the therapy takes at least six months. The length of the therapy makes patient compliance difficult; this is a frequent source of drug-resistant strains. The need for the lengthy treatment is a consequence of the presence of a population of persistent bacilli that are not effectively eliminated by the current TB drugs.

In fact, TB patients are rendered non-infectous after the first two weeks of chemotherapy; the remainder of the six-month therapy is to kill a population of slowly-metabolising persistent bacilli and to allow the host to develop protective immunity to control the residual number of bacilli not killed by the drugs. Current TB drugs are mainly active against growing bacilli, except RMP and PZA. RMP is active against both actively growing and slowly-metabolising non-growing bacilli, whereas PZA is active against semi-dormant non-growing bacilli in an acidic environment, such as in active inflammation sites in the lesions. These two agents are important sterilising drugs that significantly reduce the number of bacilli in infected tissues and shorten the therapy from 12-18 months to 6 months.[30]

### 1.2.14 Multidrug–Resistant Tuberculosis (MDR-TB)

*M. tuberculosis* is naturally resistant to many antibiotics, making treatment difficult. This resistance is due mainly to the highly hydrophobic cell envelope acting as a permeability barrier but many potential resistance determinants are also encoded in the genome. These determinants include hydrolytic or drug-modifying enzymes such as β-lactamases and aminoglycoside acetyl transferases, and many potential drug-efflux systems. Knowledge of these putative resistance mechanisms will promote better use of existing drugs and facilitate the conception of new therapies.[21]

Drug-resistant TB is a growing problem throughout the world. MDR-TB is defined as a case of TB caused by a strain of *M. tuberculosis* that exhibits resistance to the two most powerful antituberculous medications, INH and RMP. Mortality among these patients is extraordinarily high, ranging from 43% to 89%. In addition, the median interval from diagnosis to death was very short; only four to sixteen weeks.[10,15] The occurrence of MDR-TB is favoured by inappropriate management of TB. In the presence of TB which is already resistant to a single drug, therapy with that drug is ineffective. If one other drug is included in the regimen, or added to the regimen, this is equivalent to monotherapy with the potentially effective second drug. Emergence of resistance to this second drug is to be anticipated, superimposed upon the previously existing resistance to the first drug, thus multiple drug resistance emerges.[14,28]

MDR-TB is more difficult to treat than its fully susceptible counterpart, often requiring 18-24 months of therapy with four to eight different medications, including daily injection for at least six months. Food security in general is an essential consideration in the treatment of MDR-TB patients. Not only is treatment less likely to succeed if patients are malnourished, medication side-effects become much more pronounced and difficult to manage.[26]

### 1.2.15 Prevention

The history of TB strongly suggests that it can be effectively controlled by sanitary measures and improved standards of living.[15] Immunisation with a live attenuated vaccine has been used effectively in situations where TB is prevalent. The vaccinating agent known as Bacilli Calmette-Guerin (BCG) is derived from *M. bovis*. BCG vaccination is about 80% effective (the incidence of TB being one-fifth that occurring in unvaccinated persons) and last at least (10-15) years.[41] Chemoprophylaxis with INH for one year is recommended for people who have had close contact with a case of TB.[10] In the recent past, the availability of modern antituberculosis drugs appeared to promise elimination of TB. Now the impaired immunity caused by HIV infection, combined with the increasing incidence of drug resistance, is making treatment of TB more difficult to achieve.[42]

## 1.3 Dihydrofolate Reductase (DHFR)

DHFR enzyme (EC 1.5.1.3), also known as 5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase, is present in all cells and is required for normal folate metabolism in prokaryotes and eukaryotes, (fig. 5).[43]

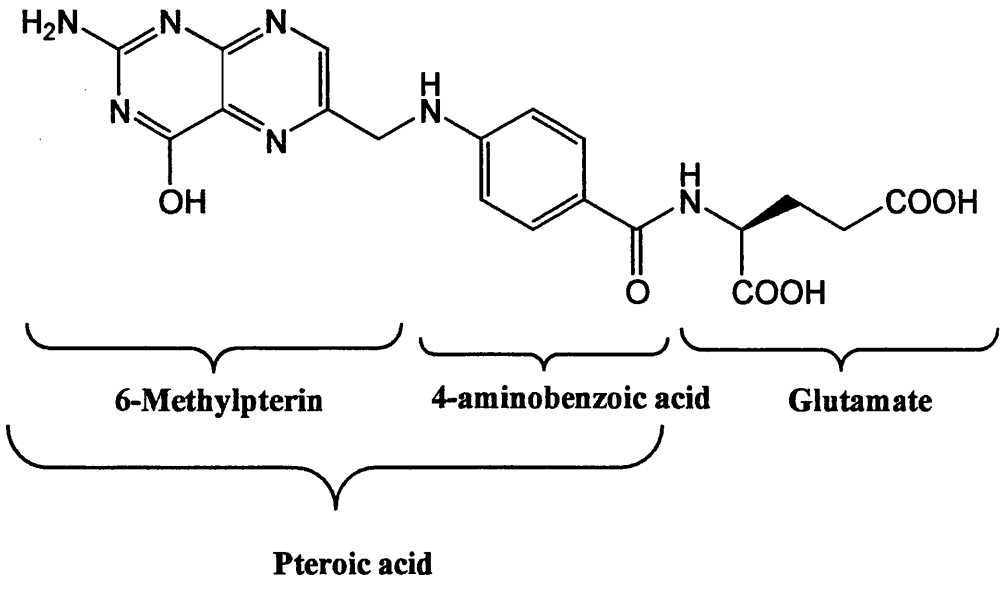
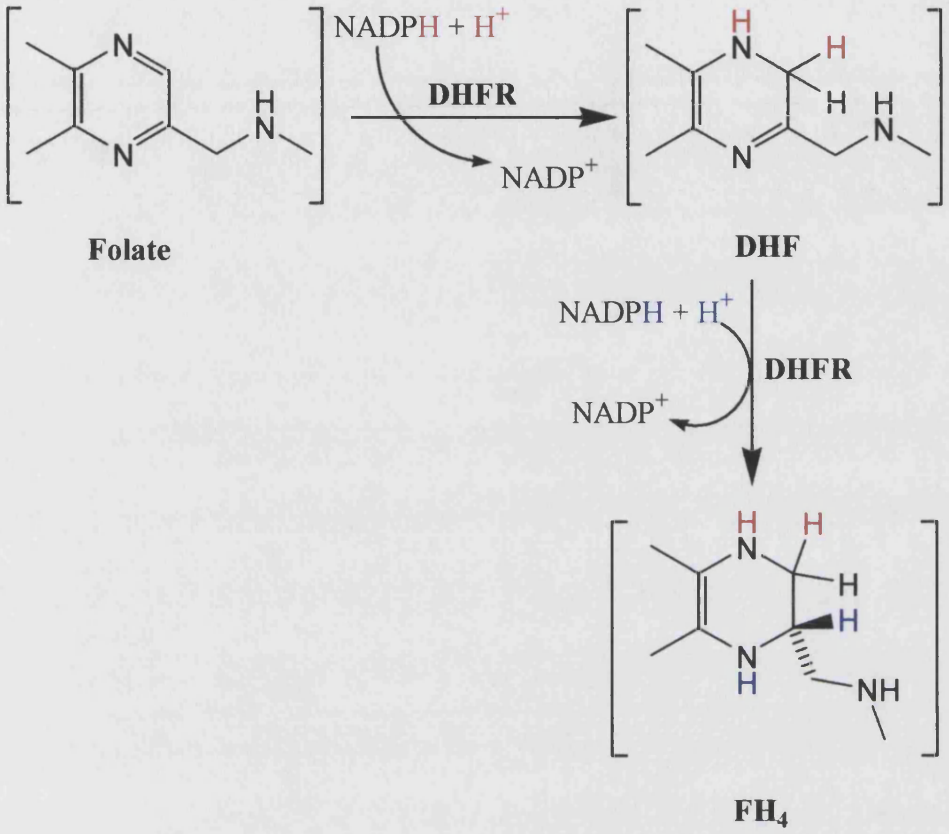



**Fig. (5) Structure of folic acid (pteroylglutamic acid)**

Inside a cell, folate is converted to the active forms by two successive reductions of the pyrazine part of the pteridine ring (scheme 2).




**Scheme (2) The catalytic function of DHFR**

Both reactions are catalyzed by DHFR using dihydronicotinamide adenine dinucleotide phosphate (NADPH) (fig. 6) as a cofactor.[44]

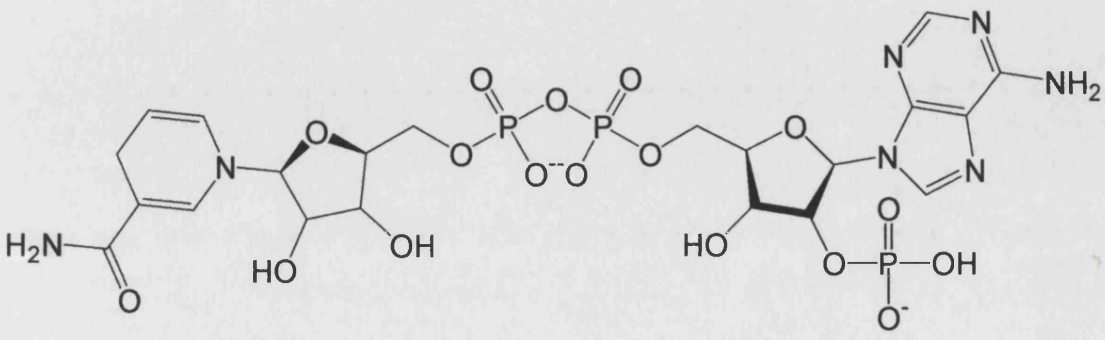


**Fig. (6) Structure of NADPH**

The first reduction yields 7,8-dihydrofolate (DHF) and the second reduction yields 5,6,7,8-tetrahydrofolate ($FH_4$). Dihydrofolate is the preferred substrate and hence its name is given to the enzyme.[34] The $FH_4$ is then converted to one-carbon adducts (fig. 7). $FH_4$ and its derivatives are necessary for the biosynthesis of thymidylate, purine nucleotides and several amino acids.[46]

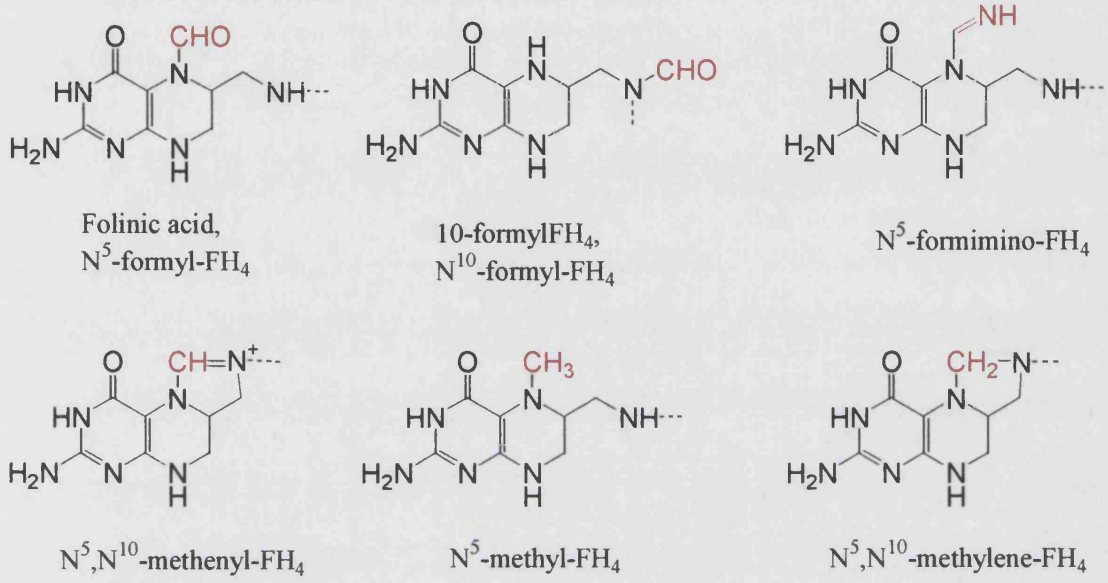


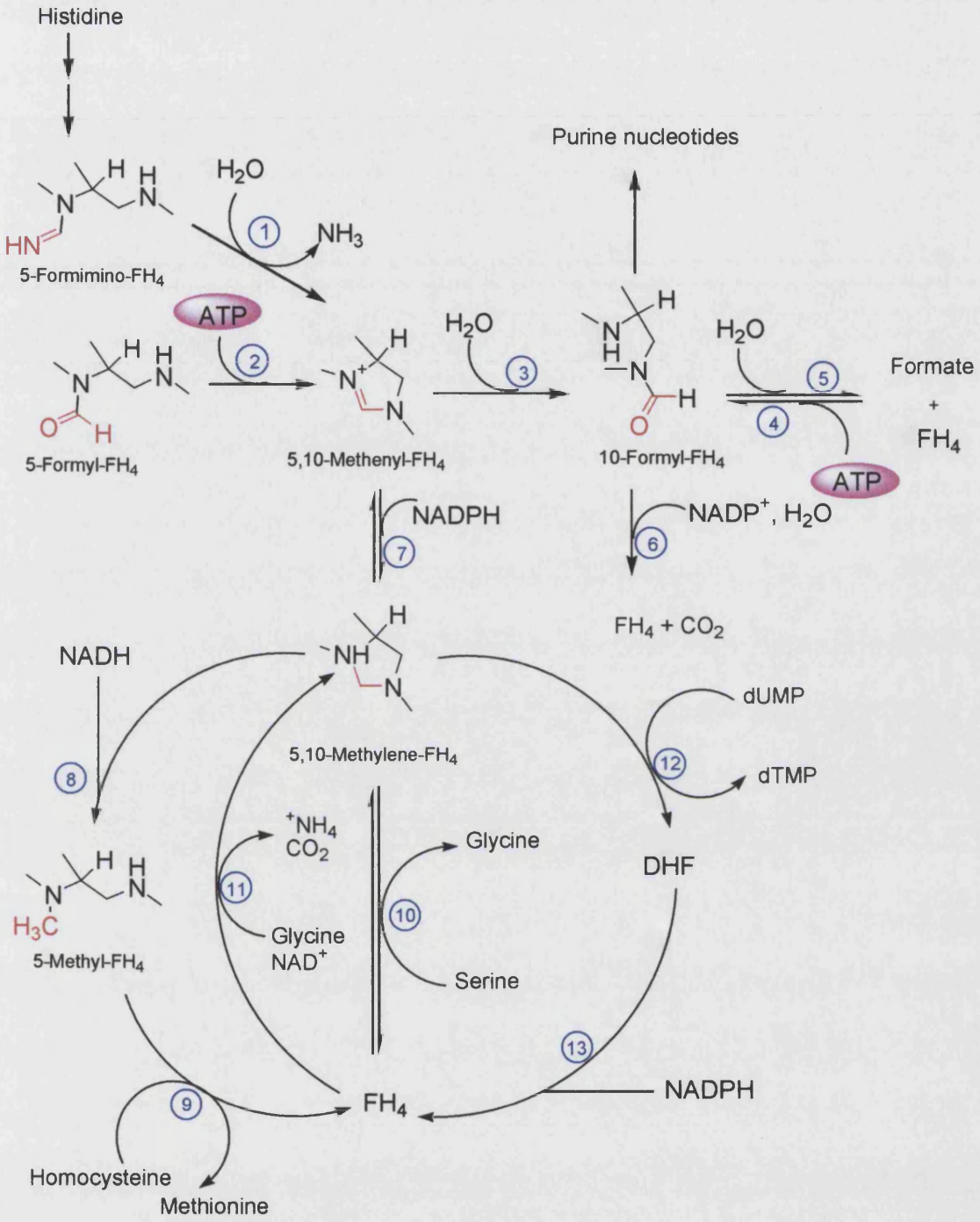

**Fig. (7) Structures of the one-carbon adducts derived from $FH_4$.**

$FH_4$ binds single-carbon units at the methyl, methylene, and formyl oxidation levels, equivalent in oxidation level to methanol, formaldehyde and formic acid respectively (fig. 7). Single-carbon groups on $FH_4$ can be carried on N-5 or N-10, or bridged between N-5 and N-10.

Formation of a cyclic bridged adduct involves dehydration, so methylene group (-$CH_2$-) is formally equivalent to a hydroxymethyl group (-$CH_2OH$) in an unbridged compound, and a formyl group (-CHO) cyclises to become a methenyl group (-CH=). In addition, there is a single-carbon adduct of $FH_4$ in which the single-carbon unit contains a nitrogen atom as well. In this formimino group (-C=NH), the carbon atom is at the same oxidation level as a formyl group. The structure of such adducts are shown in (fig. 7).[45]

The coenzymatic function of $FH_4$ is the mobilisation and utilisation of single-carbon function groups. These reactions are involved in the metabolism of serine, glycine, methionine and histidine, and in the biosynthesis of purine nucleotides and methyl group of thymine (scheme 3).

The one-carbon units carried by $FH_4$ are interconvertible. 5,10-Methylene-$FH_4$ can be reduced to 5-methyl-$FH_4$ or oxidized to 5,10-methenyl-$FH_4$. These $FH_4$ derivatives serve as donors of one-carbon units in a variety of biosynthesis. Methionine is regenerated from homocysteine by transfer of the methyl group of 5-methyl-$FH_4$.[46] Glycine yields 5,10-methylene-$FH_4$ through action of the glycine cleavage system, a multienzyme complex located in mitochondria. This reaction represents the chief catabolic route for glycine in most organisms.[45]

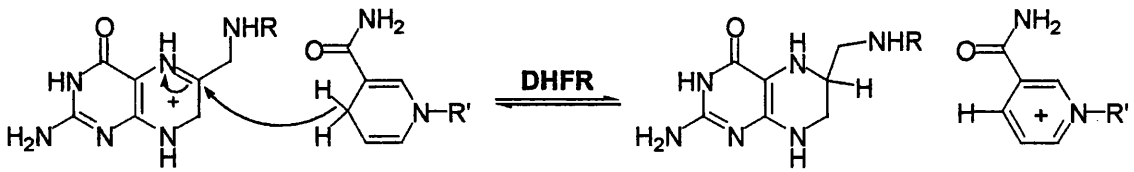



**Scheme (3) Metabolic reactions involving synthesis, interconversion, and utilisation of single-carbon adducts of $FH_4$.** The enzymes involved are (1) cyclodeaminase, (2) methenyl$FH_4$ synthase, (3) methenyl$FH_4$cyclohydrolase, (4) formyl$FH_4$ synthetase, (5) formyl$FH_4$ hydrolase, (6) formyl$FH_4$ dehydrogenase (7) methylene$FH_4$ dehydrogenase, (8) methylene$FH_4$ reductase, (9) homocysteine methyltransferase, (10) serine hydroxymethyltransferase, (11) glycine cleavage system, (12) thymidylate synthase, and (13) DHFR

The reaction of most interest in chemotherapy is the synthesis of deoxythymidylate monophosphate (dTMP) from deoxyuridine monophosphate (dUMP); this process is vitally dependent on the supply of 5,10-methylene-$FH_4$. This reaction, catalysed by thymidylate synthase, is the only *de novo* source of thymidylate, which is an essential constituent of the DNA. Therefore, inhibition of thymidylate synthesis in cells inhibits DNA biosynthesis and produces what is known as thymineless death of the cell.[46]

Thus, one-carbon units at each of the oxidation levels are utilised in the biosynthesis. Furthermore, $FH_4$ serves as an acceptor of one-carbon units in degradation reactions. The major source of one carbon-units is the facile conversion of serine into glycine, which yields 5,10-methylene-$FH_4$.

$FH_4$ is regenerated from the DHF that is produced in the synthesis of thymidylate.[45,46] This regeneration is accomplished by DHFR with the use of NADPH as the reductant.[48] A hydride equivalent is directly transferred from the nicotinamide ring of NADPH to the $C_6$ of the pteridine ring of the protonated DHF with concurrent protonation at the $N_5$ position (scheme 4).[43,45]

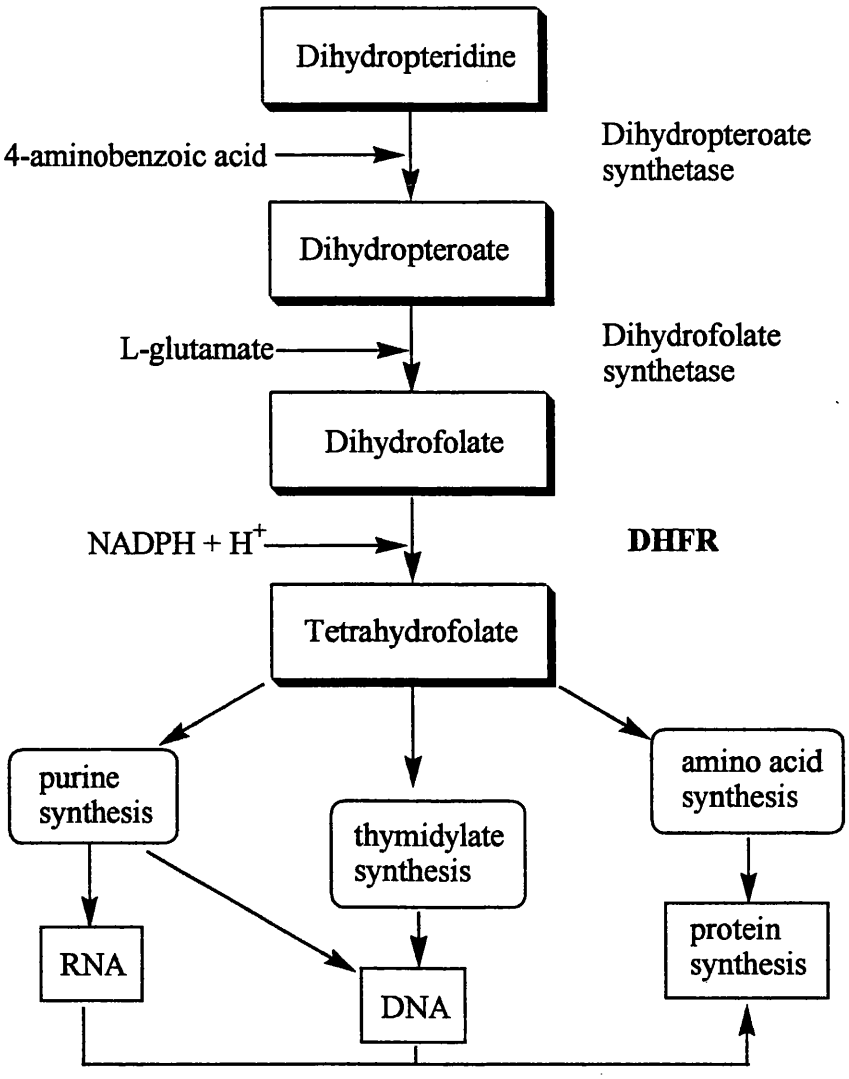



**Scheme (4) The hydride transfer reaction from the NADPH cofactor to the protonated DHF substrate.**

## 1.4 Dihydrofolate Reductase as a Target for Drug Design

There is a considerable pharmacological interest in DHFR because inhibition of the enzyme results in depletion of intracellular reduced folates necessary for one-carbon transfer reactions which, in turn, are important for the biosynthesis of

thymidylate, purine nucleotides, methionine and many other compounds necessary for RNA, DNA and protein synthesis (scheme 5).[48-51]




**Scheme (5) Biosynthesis of $FH_4$ and its role in cell metabolism**

The major effects, however, are on the biosynthesis of purines and pyrimidines, which involve one-carbon transfer reactions at several stages. The synthesis of thymine is particularly sensitive to inhibitors of DHFR because of the requirement for $FH_4$ in the transformation of dUMP to dTMP as the supply of thymine is rate-limiting in DNA biosynthesis.[52]

Microorganisms can synthesise essential $FH_4$ cofactors *de novo* with the help of dihydropteroate synthetase while mammals obtain folates from their diets or from microorganisms in their intestinal tract.[46,53] Reduction of DHF to $FH_4$ is, however a universal requirement and its inhibition leads to decrease cell growth and cell death.[43,54] When cultures of bacteria are grown in media containing amino acids and inosine, antagonism of folic acid synthesis cause the phenomenon known as thymineless death, which can be prevented by the addition of excess thymine or thymidine.

When the structure of folic acid became known, a search was made for antagonists among structural analogues of folic acid itself. These were found, but not surprisingly they were highly toxic to human cells. The toxicity of some of these compounds towards human cells is actually much greater than towards bacteria since bacterial membranes are almost completely impermeable to them.[55]

## 1.5 Inhibitors of Dihydrofolate Reductase

DHFR is the major target of drug development against several diseases such as cancer and bacterial and parasitic infections. Enzyme inhibition is effective because binding affinities of substrate analogues are so great that they are not readily displaced by the natural substrates.[48] It is also one of the best studied enzymes and the wealth of acquired knowledge is useful for selectively targeting this enzyme to design inhibitors without disrupting the function of host DHFR.[56,57]

Classical DHFR inhibitors have only a small change in structure compared to the natural substrate, so that the structure of dihydrofolic acid (fig. 8) has been utilised as a model for the design of DHFR inhibitors.[58,59] Dihydrofolic acid has been divided into four regions which have been varied in structure in many attempts to develop better inhibitors of DHFR.[56]

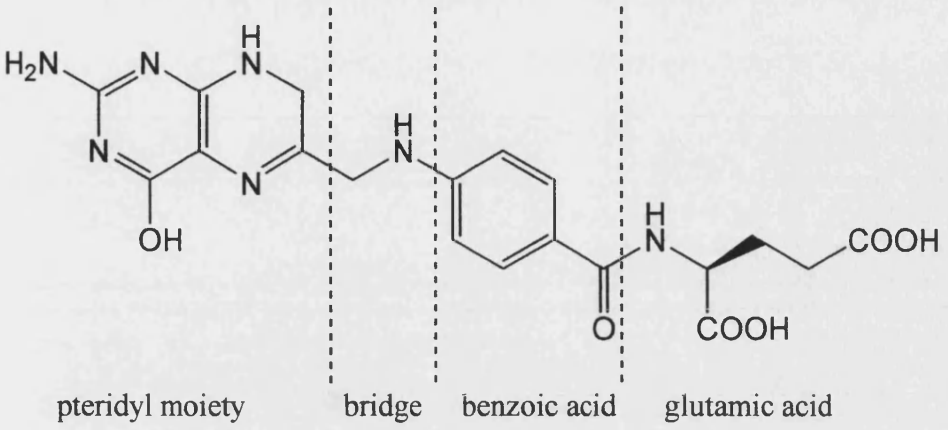



**Fig. (8) Structure of dihydrofolic acid**

The only change needed to convert dihydrofolic acid to a strong inhibitor is the replacement of 4-OH group by 4-$NH_2$ group, as well as N-methylation, as in methotrexate (fig. 9).[60,61] Methotrexate is a non-selective inhibitor employed in cancer chemotherapy. The cytotoxic action of the antifolate compound methotrexate, (fig. 9) has found a practical application in the treatment of certain malignancies, rheumatoid arthritis and psoriasis.[55,62]

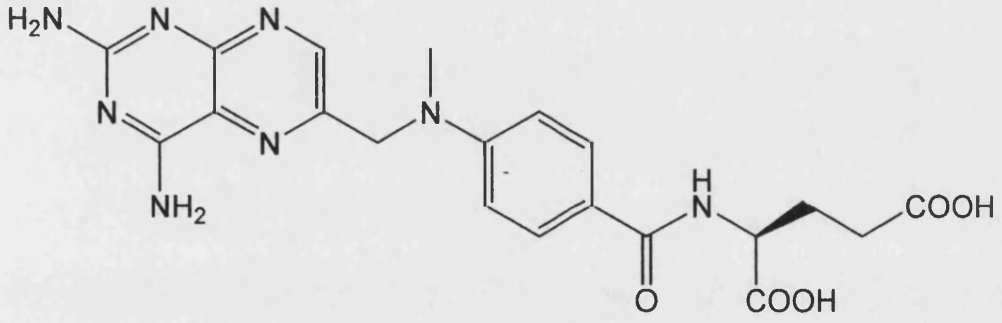
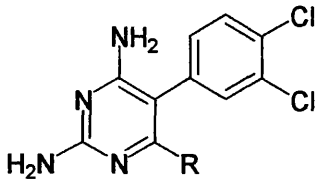


**Fig. (9) Structure of methotrexate**

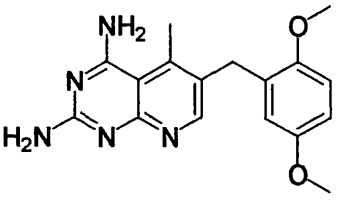
Methotrexate contains a polar L-glutamic acid moiety in the side chain and therefore requires carrier-mediated transport into cells.[64] The toxicity and narrow spectrum of activity for methotrexate, as well as the development of drug resistance, have made DHFR a target for novel antitumour and antimicrobial drug design.[65-69]

In non-classical agents, the polar side chain is replaced with a lipophilic side chain to increase cell permeability. Lipophilic DHFR inhibitors, exemplified by the first-generation antifolates metoprine (DDMP) and etoprine (fig. 10) lack a polar glutamate side chain and differ from methotrexate in not requiring a carrier-mediated active transport mechanism to gain ingress to cell, entering by passive or facilitated diffusion.[69] As a consequence, these agents exhibit activity against methotrexate-resistant tumours and also against central nervous system (CNS) malignancies inaccessible to the more hydrophilic methotrexate. The lipophilic antifolates piritrexim (PTX) and trimetrexate (TMQ) (fig. 10) were developed to overcome toxicity problems encountered with DDMP, attributed to the prolonged biological half-life and inhibition of histamine metabolism observed for this highly lipid soluble compound.[69,70]

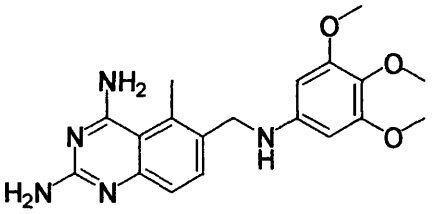
Interestingly, PTX and TMQ, but not the antimalarial antifolate pyrimethamine, are substrates for the membrane-bound P-glycoprotein (GP-170) which effluxes a diverse range of unrelated antitumour agents from cells and is responsible for the Multidrug-resistant (MDR) phenotype. Lipophilic DHFR inhibitors, including PTX and TMQ, have also enjoyed a more recent resurgence in interest as agents for the treatment of infection by opportunistic pathogens, including *Candida albicans*, *Toxoplasma gondii*, and *Pneumocystis carinii*, in immunocompomised patients.[71-74] Indeed, TMQ has recently gained clinical approval for the treatment of *P. carinii* infections in patients with acquired immune deficiency syndrome (AIDS). Unfortunately, unlike pyrimethamine or the antibacterial DHFR inhibitor trimethoprim, these antifolates exhibit no selectivity for pathogen DHFR and are more potent inhibitors for the mammalian enzyme. As a consequence, the concomitant administration of leucovorin is necessary in order to ameliorate host antifolate toxicity.[70]



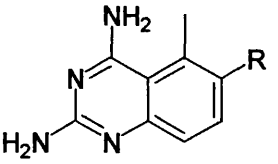
Metoprine (DDMP) : R = Me
Etoprine : R = Et



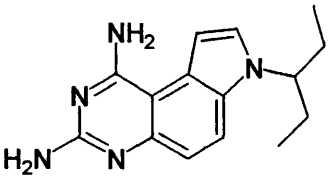
Piritrexim (PTX)



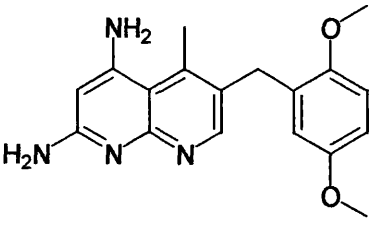
Trimetrexate (TMQ)



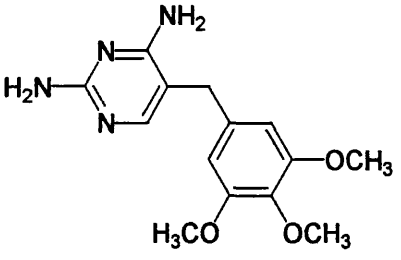
2,4-diamino-5-methyl-6-substituted quinazoline



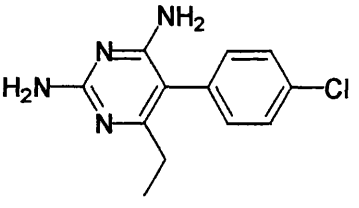
GW 345



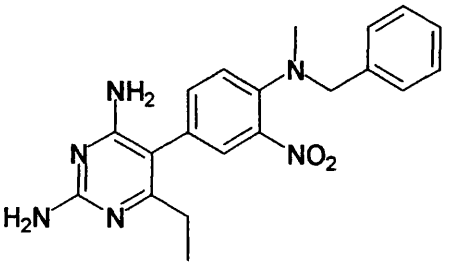
BW 301U



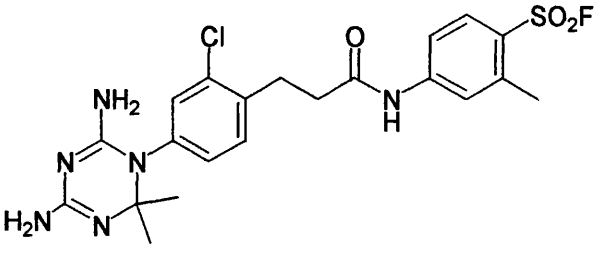
Trimethoprim



pyrimethamine



Methylbenzoprim (MBP)



2,4-Diamino-5,6-dihydrotriazine derivative

**Fig. (10) Structures of a list of non-classical DHFR inhibitors**

Non-classical DHFR inhibitors have large but appropriate structural changes compared to dihydrofolic acid. This class of inhibitors includes quinazolines, pyrimidines and triazines.[46]

### 1.5.1 Quinazolines

2,4-Diamino-5-methyl-6-substituted quinazolines (fig. 10) were found to be moderately to strongly effective inhibitors of mycobacterial DHFR. They also have significant anti-leukaemic activity.[44,75] The pyrroloquinazoline GW 345 (fig. 10) is a member of series of high-affinity inhibitors of the fungus *C. albicans* DHFR. However, this series of compounds inhibits human DHFR more strongly than it inhibits fungal DHFR.[75] Compound BW 301U (fig. 10), with a 1,8-naphthyridine nucleus, shows considerable promise as an antitumour agent.[76]

### 1.5.2 Pyrimidines

2,4-Diaminopyrimidine derivatives (fig. 10) have been tested against various forms of DHFR.[62] Although most living cells contain DHFR, the enzyme evidently differs in structure details amongst major groups of organisms, and a useful degree of species specificity in the action of inhibitors is possible. For example, pyrimethamine (fig. 10) is poorly active against the mammalian and bacterial enzymes but has an exceptionally strong affinity for the enzyme from the *Plasmodium falciparum*, which account for its specific antimalarials action.[77] The pyrimidine derivative trimethoprim is also a highly selective compound against the bacterial DHFR, (fig. 10) as reduction of the activity of bacterial DHFR by 50% requires 0.01 μM, whereas the same inhibition of the human enzyme requires 300 μM.[55,63]

These are considered the largest class of antifolate studied,[78-80] probably because trimethoprim (fig. 10) is the most potent inhibitor of bacterial DHFR to date. It has been in the public domain since 1959 and is far the most widely used antibacterial antifolate.

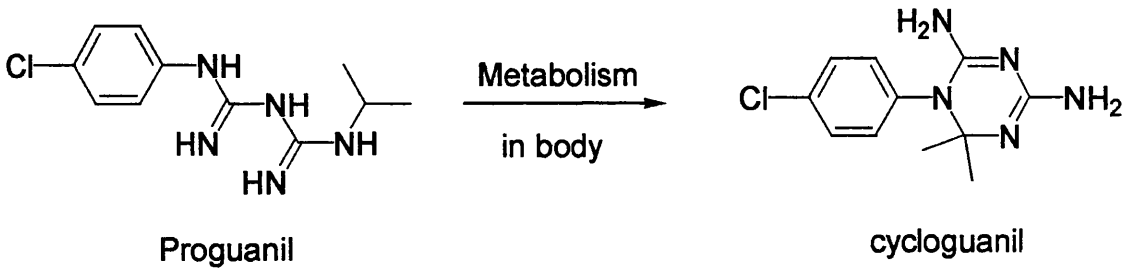
The non-classical antifolate methylbenzoprim (fig. 10) is a potent DHFR inhibitor with spectacular antitumour activity against the methotrexate-resistant

mouse M5076 reticulum cell sarcoma.[55,81] Kinetic studies with rat liver DHFR show that MBP is an inhibitor that competes with NADPH as well as DHF.[82]

### 1.5.3 Triazines

2,4-Diamino-5,6-dihydrotriazine derivatives (fig. 10) were found to interfere with folic acid metabolism.[83] Although these substances are not very active against bacterial DHFR, they have shown definite promise in cancer chemotherapy.[52,83]

Although the direct analogues of folic acid were of no value as antibacterial agents, other compounds more distantly related to folic acid have considerable importance. The potential of this type of compound was first realized in two drugs developed as antimalarials, pyrimethamine and proguanil. The latter compound is a prodrug that is metabolised in the liver to the active agent, cycloguanil (5,6-dihydrotriazine derivative), (scheme 6).[56]




Proguanil cycloguanil

**Scheme (6) Proguanil and its active metabolite cycloguanil**

Although DHFR does not represent a new target for drug design, there is still enthusiasm for the development of improved derivatives of this class of inhibitors,[84-89] particularly with regards to mycobacteria.[90-92] A unique feature of DHFR is the selectivity that is possible in the design of inhibitors; this makes it an ideal old target for rational and effective drug design for antimycobacterial agents.[52,65]

As DHFR has been studied extensively, especially in the last few years, more detailed insight into the structure and mode of action of the enzyme has been gained

through the X-ray determination of enzyme isolated from different species. The three-dimensional structures of the enzyme became available at the same time as molecular graphics emerged as a new tool in drug design. Together, they allowed a new approach to the design of novel inhibitors of DHFR.[50]

## 1.6 DHFR Structure and Ligands Binding Site

In the past 20 years, considerable advances have been made in the understanding DHFR catalysis and inhibitor binding and the most useful information has come from correlating physical and kinetic properties with amino-acid sequences and protein conformations. The amino-acid sequences of DHFR from numerous bacterial and vertebrate sources are known.[54,93,94]

In general, there is much greater homology (76%) among the DHFRs from vertebrate sources than there is among the bacterial reductases (13%). DHFR has been classified as a doubly wound mixed β-sheet in which the central eight-stranded β-sheet is protected on either side by two α-helices. The individual strands of the β-sheet are designated A-H in order of their occurrence in the linear protein sequence. Each helix is assigned the letter of the β-strand that it precedes in the sequence.

A deep cleft exist on the enzyme surface between α-helices B and C and this cavity serves as the binding site for the diaminopyrimidine-type inhibitors and presumably the substrate dihydrofolic acid. The nicotinamide portion of the cofactor also binds in this cleft, in a position to transfer its hydride equivalent to the adjacent substrate. The diphosphate bridge of the cofactor is draped over the edge of the β-sheet in a shallow groove between the amino termini of the α-helices C and F, and the adenine moiety binds in a niche on the other side of the sheet.[93]

### 1.6.1 Three-dimensional Structure of *M. tuberculosis* DHFR and Ligands Binding Site

Recently the three-dimensional structure of *M. tuberculosis* (Mtb) DHFR reveals that the central β-sheet of Mtb-DHFR consists of seven parallel strands and a single C-terminal antiparallel strand (fig. 11).[83]

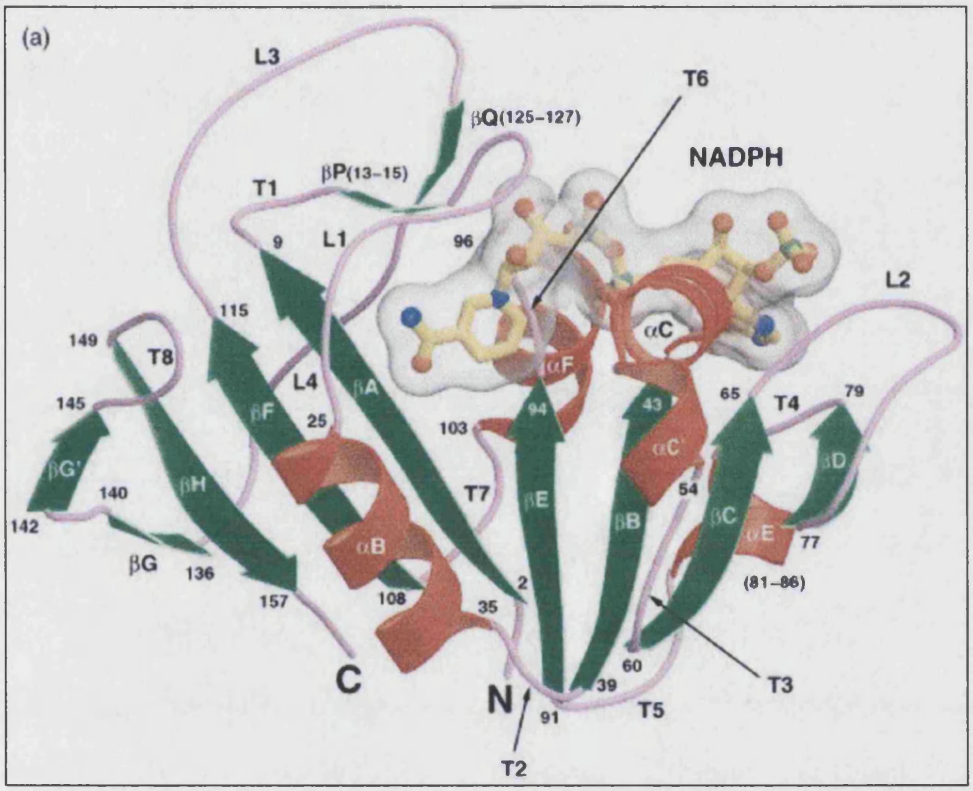



**Fig. (11) Schematic ribbon diagram of the overall fold of Mtb-DHFR**

α-Helices are in red and β-strands in green. NADP is in a ball-and-stick model with its molecular surface being transparent. Colour code for atoms: carbon in yellow, oxygen in red, nitrogen in blue and dark green for phosphorus.

The β-sheet has a left-handed twist of about 130°. In the holoenzyme, the coenzyme NADP is bound in an extended conformation by the C-terminal part of the β-sheet, with the nicotinamide ring inserted into a cleft formed by strands β-A and β-F.

NADP interacts extensively with the protein. The pyrophosphate moiety of NADP is interacting favourably with helix dipoles by binding near to the amino ends of helices α-C and α-E. The pyrophosphate also forms a salt bridge with Arg45. The adenine ring contacts the protein through interactions involving two residues Leu65 and Leu102 on one side, and through stacking interactions with the side chain of Arg67 in the other side. In addition, the adenine ring contacts several other residues. While the adenosyl ribose loosely interacts with protein its O2'-phosphate interacts strongly with Mtb-DHFR through five H-bonds. The nicotinamide ribose only contacts protein residues in loop L1, while the nicotinamide ring interacts with residues from strands β-A, β-E, β-I and loop L1.[83] In the ternary complexes, each containing NADP plus an inhibitor, the conformation of the cofactor is essentially identical with that of binary complex. Also, the interactions of NADP with protein residues are very similar.

Enzyme inhibitors such as methotrexate (MTX) and trimethoprim (TMP) bind essentially in the same pocket and their nitrogen-containing heterocyclic rings position in the same orientation. A common feature of the binding mode is a set of H-bonds between the heteroatomic rings and several structurally conserved residues. The other moieties of inhibitors bind to quite different protein residues.[83]

### 1.6.2 Overall Structural Comparison of Mtb-DHFR and Human DHFR

The overall structural comparison of Mtb-DHFR and human DHFR reveals that Mtb-DHFR contains 159 amino acid residues, compared with 187 for the human protein with a sequence identity of 26%. A superposition of the enzymes from pathogen and host shows that the general fold of Mtb-DHFR is essentially the same as that of the human protein, even though the human enzyme is significantly larger. Structural differences in the active site of DHFR between Mtb and human enzyme are of particular interest for the design of therapeutic compounds that would selectively bind to the Mtb protein and would not affect the function of the human DHFR. The sequence identity in the active and ligand binding site is significantly higher, about 55%, than the sequence identity of 17% for the reminder of the chain but some aspects of the ligand binding area of Mtb-DHFR appear to differ in a significant and promising way from that in the human enzyme.

These key differences make them suitable to be exploited for the design of antituberculous specific compounds.[83,93]

### 1.6.3 Structural Basis for Selectivity

The structure of Mtb-DHFR has been determined alone and in complexes with NADP and with the inhibitors such as MTX and TMP. Detailed analysis of the interaction of human DHFR and Mtb-DHFR with cofactor NADP and with inhibitors shows important differences relevant for drug design.[83]

The key differences in the active site between the Mtb-DHFR and human enzyme are: first, the presence of a glycerol (A) molecule that is found in a depression near the folate binding site. This glycerol molecule is bonded through hydrogen bonds with the side-chains of Trp22 (indole nitrogen is H-bond donor), Asp27 (carboxylate is H-bond acceptor) and Gln28 (amide carbonyl is H-bond acceptor) which form a pocket in Mtb-DHFR. In contrast, in the human DHFR complexes containing folate or MTX, this glycerol A site is well packed with three hydrophobic residue side-chains of Leu22, Pro26 and Phe31. Second, two small hydrophobic residues in Mtb-DHFR, Pro25 and Ala29, correspond to two large positively charged residues, Arg28 and Arg32, in the human enzyme while one negatively charged residue, Glu33 in Mtb-DHFR is changed into a positively charged Arg36 in the human enzyme.[83]

# 2. AIMS AND OBJECTIVES

## 2.1 Aims

DHFR has distinct advantages as a potential drug target. Firstly, the biochemistry of the folate pathway and DHFR enzyme are well characterised and the crystal structure of Mtb-DHFR has been recently solved. Secondly, this long history means that the safety and selectivity of these inhibitors have been intensively studied.

The aim of this research project was to explore the active site of Mtb-DHFR looking for features for selectivity and potency and accordingly design and synthesis *novel* selective Mtb-DHFR inhibitors. Selective inhibitors will provide lower doses, shorter schemes for treatment, minimal side-effects and consequently reduce the number of MDR-TB cases.

## 2.2 Objectives

The objectives of this research project are as follows:

1- Study the active site of Mtb-DHFR and compare it with the corresponding enzyme in the human.
2- Design a new functional group to increase the selectivity of 2,4-diaminopyrimidine nucleus as inhibitors towards Mtb-DHFR.
3- Modify the structure of the newly designed functional group in order to explore its structural and stereochemical requirements for selectivity as Mtb-DHFR inhibitors.
4- Synthesis the designed group of compounds.
5- Evaluate the synthesised compounds for potency and selectivity.

# 3. DISCUSSION

## 3.1 Potency and Selectivity of DHFR Inhibitors

One of the most important questions in antifolate chemotherapy concerns the tremendous selectivity of trimethoprim for bacterial enzymes compared to vertebrate enzymes.[95,96] Trimethoprim is over 100,000 times as potent an inhibitor against *E. coli* DHFR compared with human DHFR.[95] What factors determine the differences in substituent effects between the two enzymes? Why is the design of selective bacterial antifolates with minimal mammalian toxicity so cumbersome, complex and difficult?[97]

In an early analysis of the problem, the inhibition of DHFR by benzylpyrimidines (fig. 12) constitutes one of the most thoroughly studied QSAR (quantitative structure activity relationship). In a SAR study of rat liver DHFR, Hyde and Roth realised that the hydrophobic interactions of the substituents were of paramount importance while, with *E. coli* DHFR, only steric factors came into play.[95]

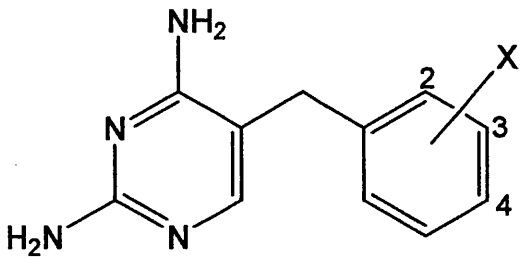



**Fig. (12) Substituted 2,4-diamino-5-benzylpyrimidines**

It is not easy to select the best derivatives from the point of view of maximizing potency in one system while minimizing it in another, even with the QSAR for the two systems. The crux of the matter in the present case is that, in order to make more bacterial antifolates, one needs to synthesise unsymmetrical benzylpyrimidines with a lipophilic substituent in the 3-position and a polar

substituent in the 5-position. The more polar the substituent at 4-position is, the better the selectivity. However, polar groups also tend to reduce antibacterial as well as antiavian potency.[97] It is not surprising, then, that obtaining more effective *novel* inhibitors calls for making unusual derivatives.[98]

### 3.2 Molecular Graphics-Based Drug Design

QSAR studies have relied heavily on the use of computers from the beginning for statistical calculations involving multiparameter equations. Researchers soon realised that drug design could be aided significantly if structures of receptors and drugs could be displayed on computer terminal and molecular processes could be observed.

Molecular graphics is the visualisation and manipulation of three-dimensional representations of molecules on a graphics display device. The potential to apply this technology to protein crystallography was quickly realised and by early 1970s electron density data from X-ray diffraction studies could be presented and manipulated in stick or space-filling multicolour representations on a computer terminal.[99] The number of X-ray crystal structures available in the Protein Data Bank (PDB) went from about 200 in 1990 to more than 31,000 by 2005.[99,100]

A three-dimensional computer graphics representation of a protein that can be manipulated in three dimensions allows the operator to visualise the interactions of small molecules with biologically important macromolecules. Superimposition of structures can be performed easily by molecular graphics. The most effective use of molecular modeling is when a high-resolution crystal structure of a receptor with a ligand bound is available. Molecular graphics visualisation of the electron density map of this complex may reveal empty pockets in the complex that could be filled by appropriate modification of a lead compound.[99]

Numerous molecular graphics systems are available, but the typical system consists of a mainframe or supermini computer linked to a high-resolution graphics terminal with local intelligence. The mainframe or minicomputer executes all of the molecular calculation of bond lengths and bond angles. One of these systems which

we have used on my research is the Silicon Graphics INDY together with the Midas Plus program.[101]

The molecular Interactive Display and Simulation (MIDAS) System is a collection of programs developed by the Computer Graphics Laboratory at the University of California, San Francisco (UCSF). The major component of the MIDAS system is an interactive graphics display program. Midas Plus™, designed for the display and manipulation of macromolecules such as proteins and nucleic acids. Midas Plus is capable of displaying molecular structures and surfaces from information contained in PDB format files.[102]

### 3.3 Design of the Target Compounds

The three-dimensional structure of Mtb-DHFR was recently studied and became available from the PDB. Three crystal structures of ternary complexes of Mtb-DHFR with NADP and different inhibitors as trimethoprim (TMP) and methotrexate (MTX), have been determined as well as the binary complex with NADP.[83]

Structural comparison of these complexes with the human DHFR reveals clearly two major avenues for arriving at *novel* selective inhibitors of Mtb-DHFR; one involves the exploitation of differences in side-chain; a second avenue involves the glycerol A pocket of Mtb-DHFR which is essentially absent in the human enzyme. The glycerol A pocket refered to the presence of a glycerol molecule bound to a cavity close to the N8 position of MTX (fig. 13) and the aminopyrimidine ring of TMP. In contrast, in the human DHFR complexes containing folate or MTX, this glycerol A site is well packed with three hydrophobic residue side-chains, Leu22, Pro26 and Phe31.[103,104]

Moreover there are two residues, Leu22 and Phe31, adjacent to the N8 and C7 of MTX in the human DHFR, which restrict the accessibility of MTX to solvent, with the distance between the side-chains of Leu22 and Phe31 being about 3.8 Å. These two residues in the human enzyme correspond to residues Leu20 and Gln28 in Mtb-DHFR where the shortest distance between side-chain atoms is about 6.5 to 9.4

Å. Consequently, a MTX analogue with an additional group connected to N8 or C7, and incorporating features of binding mode of the glycerol molecule to Mtb-DHFR active site, may bind selectively to Mtb-DHFR as such a MTX-derivative should be sterically and chemically hindered from forming a complex with human DHFR.

It was found that the glycerol molecule in the active site of Mtb-DHFR is bound to the protein through three hydrogen-bonds between all the hydroxy groups and residues Asp27, Gln28 and Trp22. The glycerol carbon atoms are in hydrophobic contact with the side-chain of Leu20. Consequently the conformation of the glycerol molecule is fixed on a definite conformation (fig. 13) that could be studied and used as a template for designing a new function group that could form hydrogen-bonds with the hydroxy groups of the glycerol molecule.[83]

The conformation and the binding mode of the inhibitors MTX and TMP in ternary complexes revealed that both bind essentially in the same pocket and their nitrogen-containing heterocyclic rings position in the same orientation. A common feature of the binding mode is a set of strong hydrogen-bonds between the heteroatomic rings and several structurally conserved residues. The other moieties of inhibitors bind to quite different protein residues.

Since the inhibitors MTX and TMP bind in a similar fashion to the active site, the glycerol A pocket near N8 of MTX is also in immediate neighbourhood of TMP. This suggests that not only MTX but also TMP can be extended by substituents which bind in the glycerol A pocket. These extensions to the common diaminodiazine motif which contributes the affinity of the inhibitors could increase the selectivity of the new inhibitors over human DHFR. Clearly, such diaminodiazine variants would have very different chemical structures than compounds derived from MTX, increasing therefore the likelihood that *novel* and highly selective inhibitors of Mtb-DHFR can be obtained.

Significant differences were also observed near to the glutamate moiety of MTX. Two small hydrophobic residues in Mtb-DHFR, Pro25 and Ala29, correspond to two large positively charged residues, Arg28 and Arg32 in the human DHFR. These differences are also favourable for design of selective inhibitors, as Pro25 and

Ala29 in Mtb-DHFR allow for favorable interactions with hydrophobic substituents of new inhibitors, at the same time, these hydrophobic substituents are likely to decrease significantly the affinity of the inhibitors for human DHFR.[83]

Aiming to develop such selective Mtb-DHFR inhibitors, we studied the X-ray crystal structure of the binary complex of MTX and Mtb-DHFR to explore the relationship between the MTX and the glycerol pocket at the active site. By the aid of Silicon Graphics INDY and using the Midas Plus program we could determine the conformation of the glycerol molecule at the active site (fig. 13) and could measure the distance between MTX molecule and the glycerol molecule (fig. 14) which in turn helped us to determine the appropriate configuration and length of the side chain that should fit perfectly into the glycerol pocket.

It is reported that the best known inhibitors of DHFRs include methotrexate, trimethoprim and pyrimethamine, which to a different extent discriminate between DHFRs from eukaryotic and prokaryotic organisms. Methotrexate is more potent inhibitor to the mammalian DHFR, while trimethoprim and pyrimethamine are potent inhibitors of bacterial and protozoal DHFR, respectively, but only weak inhibitors of the mammalian DHFRs.[83] Pyrimethamine had shown some activity against *M. avium* complex.[105] Trimethoprim was reported to have poor activity against mycobacteria.[106]

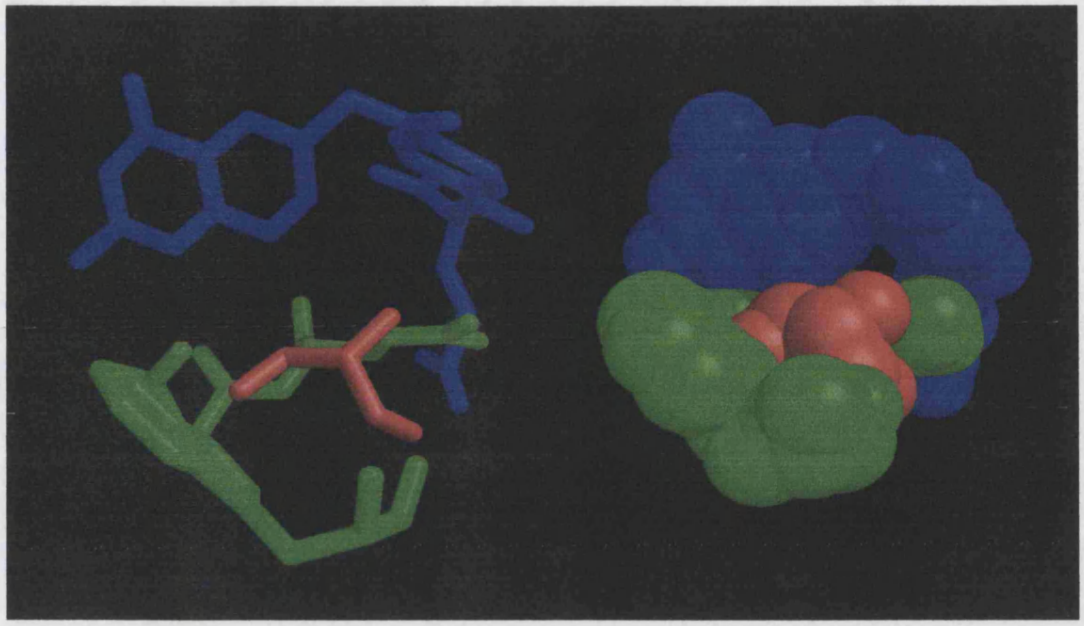


**Fig. (13) Stereoview of the glycerol molecule (red) and the glycerol pocket A in the active site of Mtb-DHFR in MTX (blue)-DHFR binary complex (left) and in space-filling (right)**

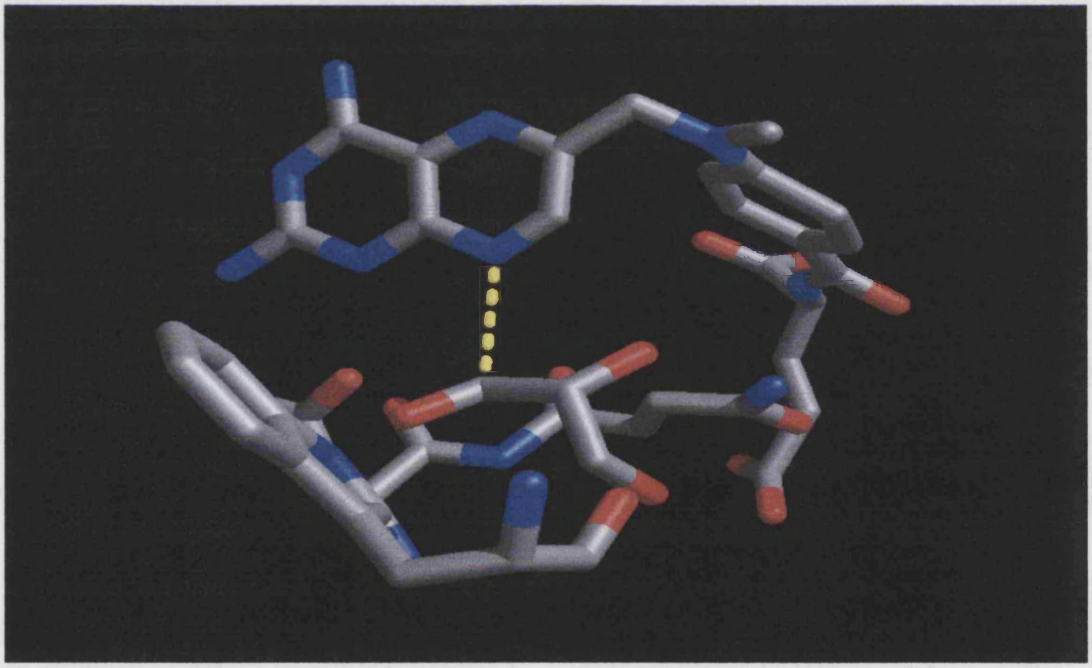


**Fig. (14) Measurement of the distance between the glycerol molecule and MTX in the active site of Mtb-DHFR, carbon on grey, oxygen on red and nitrogen on blue**

Being aware of the crucial contribution of 2,4-diaminopyrimidine pharmacophore to the DHFR binding together with our study to the active site, we decided to integrate this information into a design of *novel* compounds to be synthesised and tested as selective Mtb-DHFR inhibitors.

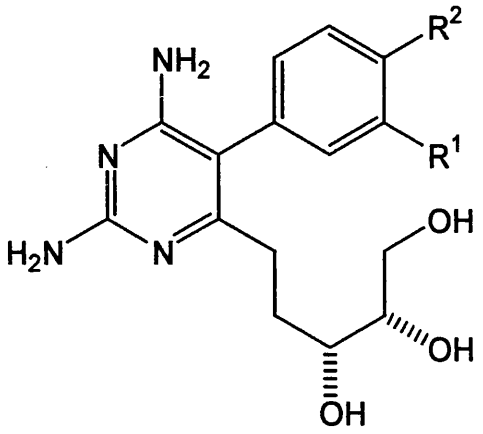
Accordingly our target compounds would contain the 2,4-diaminopyrimidine nucleus with an unique extension at 6-position that would provide strong binding to the glycerol A pocket and a hydrophobic group at 5-position such as a substituted phenyl group could bind to the hydrophobic residues on Mtb-DHFR and decrease significantly the affinity of these *novel* inhibitors to the human DHFR. The new side-chain at the 6-position was constructed depending on our study to the conformation of the glycerol molecule at the active site (fig. 13) and measuring the distance between N8 of MTX and the glycerol A pocket which was 2 Å (fig 14) at MTX-Mtb-DHFR ternary complex.

Firstly, the new functional group should be 2 Å away from the N8 of MTX molecule. As the bond length of the C-C ($sp^3$-$sp^3$) bond is 1.54 Å and by considering that the tetrahedral bond angle is 109.5°,[107] accordingly the designed functional group should have two carbon bond lengths apart from the N8 of MTX or three carbon bond lengths apart from the pyrimidine ring.

Secondly, the new function group should contain three substituents such as hydroxy group that could form hydrogen-bonds with the glycerol molecule at the active site of Mtb-DHFR. Finally the configuration of these three hydroxy groups should resemble the fixed conformation of the glycerol molecule in the active site to provide perfect binding conditions.

Consequently, the (3*R*,4*S*)-3,4,5-trihydroxypentyl group was the best extension at the 6-position of the pyrimidine ring that would satisfy these requirements and should provide the desired selectivity for our target compounds. The designed target compounds (fig. 15) would contain the 2,4-diamino-5-(substituted phenyl)pyrimidine nucleus with the new side-chain, (3R,4S)-3,4,5-trihydroxypentyl, at the 6-position to bind to the glycerol pocket in Mtb-DHFR and should provide steric hindrance that could prevent these compounds from binding to

the human DHFR. Furthermore, different substituents at the phenyl group at 5-position also were designed to explore their effects on the activity and selectivity as DHFR-inhibitors.




**$R^1$ = H, Cl, $NO_2$**
**$R^2$ = H, Cl, Br, $NCH_3Bn$**

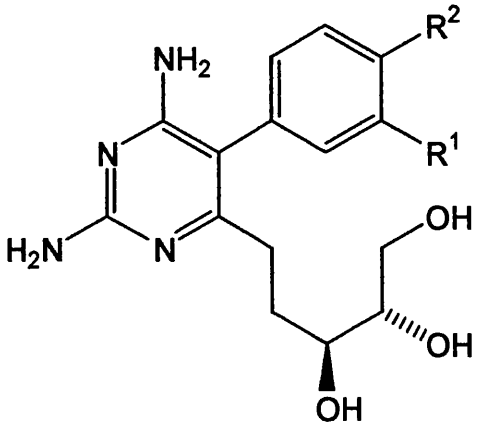
**Fig. (15) Target compounds group (A)**

There are five hydrophilic groups already present on this group A target compounds (fig. 15) and in order to increase the hydrophobicity of such compounds we considered the introduction of lipophilic substituents such as chlorine, bromine and $NCH_3Bn$ group to the phenyl side-chain aiming to increase the probability of the target compounds to pass the lipophilic mycobacterial membrane.

The introduction of an electron-withdrawing group, such as the nitro group, *ortho* to the halogen was essential chemically to increase the activity of the benzene ring towards the nucleophilic substitution of the halogen with secondary amine. The designed target compounds group A (fig. 15) should accommodate in the active site of Mtb-DHFR with the new functional group at 6-position that resembles the structure and configuration of the glycerol molecule located in the active site of Mtb-DHFR. That new function group should provide a strong binding of the *novel* inhibitors group A to the glycerol pocket through strong hydrogen-bonds as the configurations of three hydroxy groups on the side chain were designed to be perfectly fitted in the glycerol pocket.

To understand the steric requirements of the hydroxy groups of this side-chain at 6-position, we designed another group of *novel* compounds group B (fig. 16) which would have the same nucleus as (group A) apart from the configuration of the chiral centres.

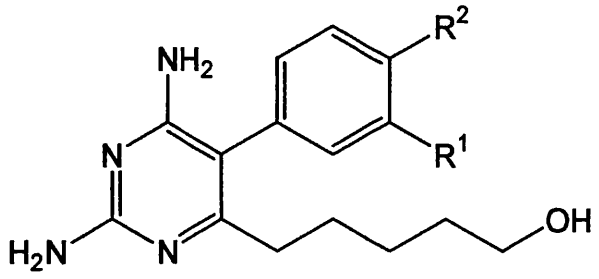
The side-chain for group B target compounds at 6-position would be the (3*S*,4*S*)-3,4,5-trihydroxypentyl group. We anticipate that this side-chain should not fit as well into the glycerol A pocket, compared to group A target compounds. Consequently group B target compounds should be less potent than group A compounds. On the other hand, it should not affect the selectivity of group B compounds as there are still two hydroxy group that should bind probably to the glycerol A pocket in Mtb-DHFR and provide steric hinderance for binding to the human DHFR.




**$R^1$ = H, Cl, $NO_2$**
**$R^2$ = H, Cl, Br, $NCH_3Bn$**

**Fig. (16) Target compounds group (B)**

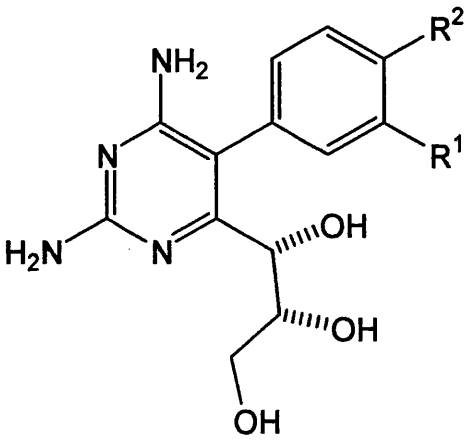
Furthermore, we decided to preserve the same carbon skeleton of the side-chain at the 6-position but with only one primary alcohol functional group to explore the importance of the deleted hydroxy-groups on potency and selectivity which could be represented by designing group C (fig. 17) as *novel* inhibitors.




**$R^1$ = H, Cl, $NO_2$**
**$R^2$ = H, Cl, Br, $NCH_3Bn$**

**Fig. (17) Target compounds group (C)**

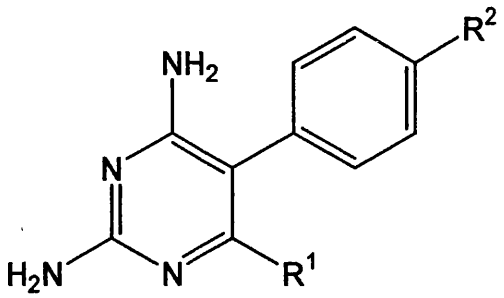
Finally, to establish the predictive value of the design of our target compounds, group D (fig. 18) was designed to have the same side chain as group A but with a shorter carbon chain at 6-position to assess the importance of the measured distance between the main nucleus and the glycerol pocket at the active site of Mtb-DHFR. Accordingly, the side chain at 6-position would be (1*S*,2*R*)-1,2,3-trihydroxypropyl group.




**$R^1$ = H, Cl, $NO_2$**
**$R^2$ = H, Cl, Br, $NCH_3Bn$**

**Fig. (18) Target compounds group (D)**

Three model compounds of substituted 2,4-diaminopyrimidines (fig. 19) with three different side-chains would also be synthesised to be compared with the activity of the target compounds.




$R^1 = CH_3, C_2H_5, CH_2CH_2Ph$
$R^2 = H, Cl$

**Fig. (19) Model compounds**

The importance of these parameters for potency and selectivity can be conveniently determined by microbiological examination of the compound in question on Mtb-DHFR and the human enzyme as well.

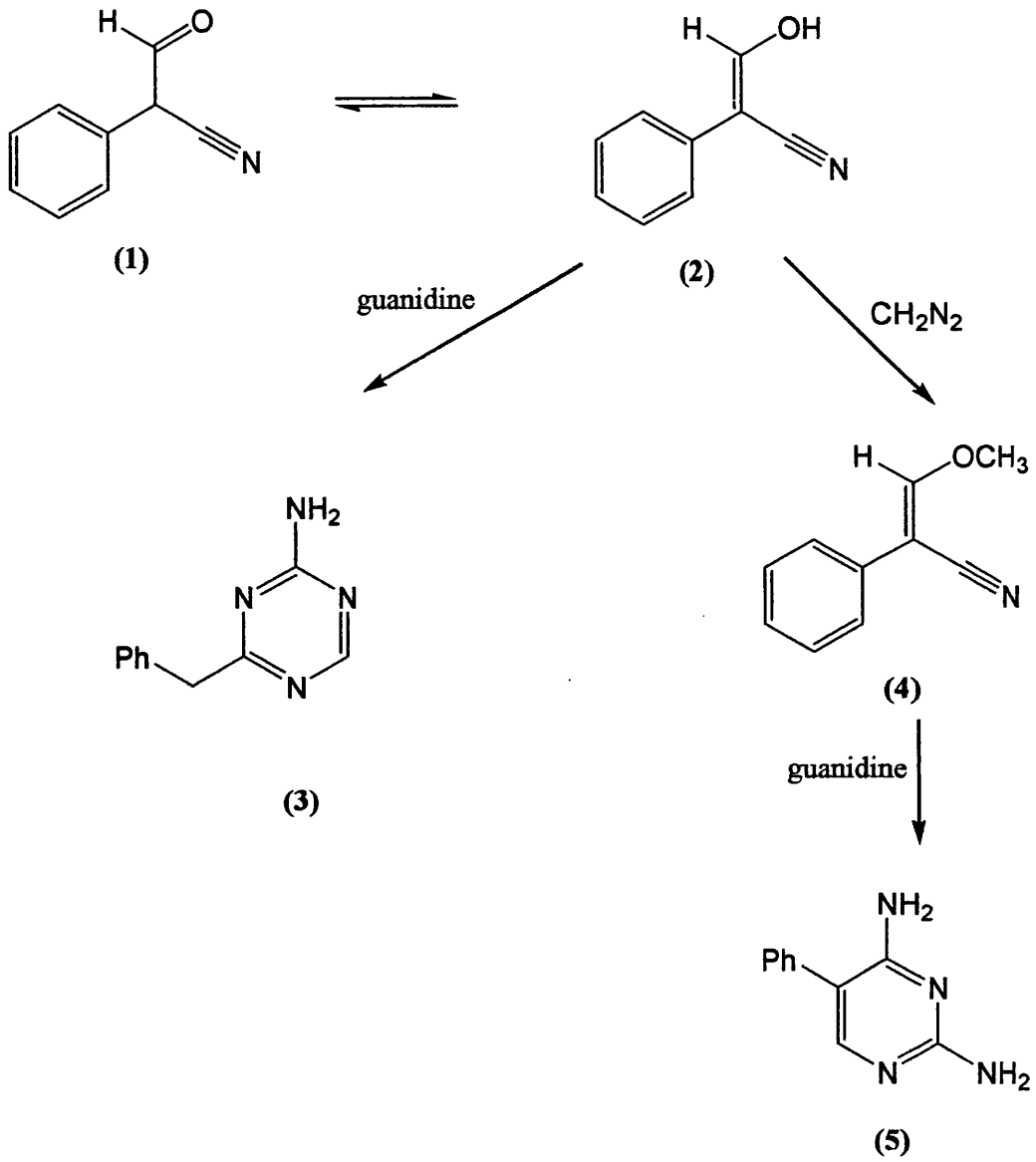
Toward this end the synthesis of the target compounds was carried out and biological examination was performed.

### 3.4 Strategies for Preparation of 2,4-Diaminopyrimidine Derivatives

Different strategies have been employed for the synthesis of different derivatives of 2,4-diaminopyrimidine.

By the principal synthesis, a primary amino group results in position 2 when guanidine is used as the one-carbon fragment in synthesis of the 2,4-diaminopyrimidine nucleus, while the primary amino group in position 4 and/or 6 arises by using a nitrile or dinitrile as a three-carbon fragment.[108] However, β-aldehydonitriles such as formylphenylacetonitrile (1), condensed with guanidine to

give 2-amino-4-benzyl-1,3,5-triazine (3) rather than the expected 2,4-diamino-5-phenylpyrimidine (5) (scheme 7).[109]

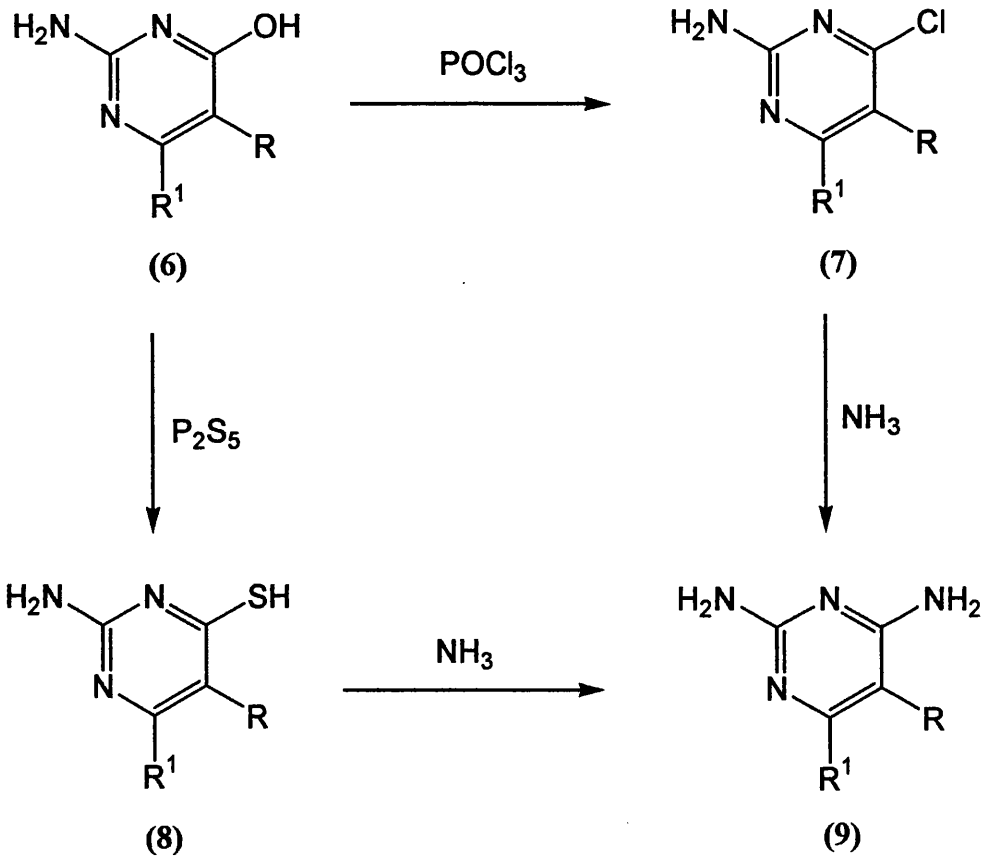



**Scheme (7) Reaction of formylphenylacetonitrile with guanidine**

The reason for the anomalous reaction of these formyl intermediates may possibly be due to their existence mainly as enols (2) which might well have too high an acidic strength for normal condensation but, when they were first converted with

diazomethane to enol ethers (4), they readily condensed with guanidine to give the expected pyrimidine (5) (scheme 7).[110]

Different strategies have been employed for the preparation of 2,4-diamino-5-phenylpyrimidine derivatives. For example α-formyl-β-phenylesters, in general, would condense with guanidine to give 2-amino-4-hydroxypyrimidine (6) derivatives which could be converted to the corresponding 2,4-diaminopyrimidine (9) derivatives *via* 4-chloropyrimidine analogues (7),[111,112] although an alternative route *via* the 2-amino-4-mercaptopyrimidine (8) may be used (scheme 8).[113,114]

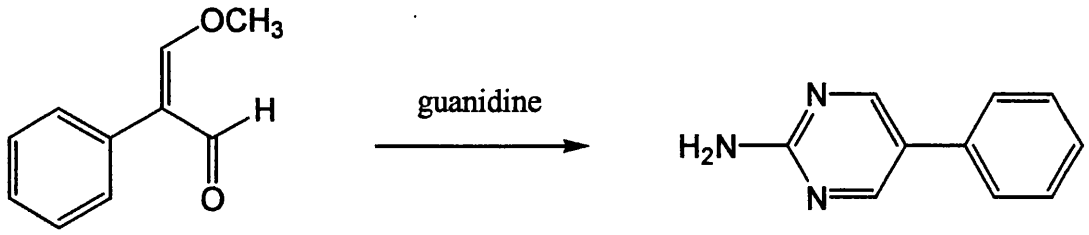



**Scheme (8) Preparation of 2,4-diaminopyrimidine derivatives from the 2-amino-4-hydroxypyrimidine analogues**

The systematic synthesis of 2,4-diaminopyrimidine derivatives *via* the 2-amino-4-hydroxy and 2-amino-4-chloropyrimidines proved to be very

unsatisfactory. Although the 5-phenyl and the *meta* and *para* chloro and bromophenyl derivatives could be prepared by this route, the yields of the aminohydroxypyrimidines were exceptionally poor. Moreover, the condensation of α-formyl-β-phenylester and guanidine failed entirely with ortho-substituted phenyl derivatives, when the aromatic nucleus was substituted by electron donor groups and with α-phenyl-β-ketoesters. The last limitation was considered the most serious.[113]

A number of possible alternative methods of synthesis involving modification of urea derivatives and esters were examined. However, several derivatives of α-acetylphenylacetonitriles failed under a variety of conditions to condense with different urea derivatives to give 2,4-diaminopyrimidines. it seemed probable that the high degree of enolisation and acidity of these β-carbonyl derivatives was fundamentally responsible for their failure to condense.[110] Rupe has overcome this difficulty by condensing guanidine with enol ether (10) to give 2-amino-5-phenylpyrimidine (11) (scheme 9).[115]

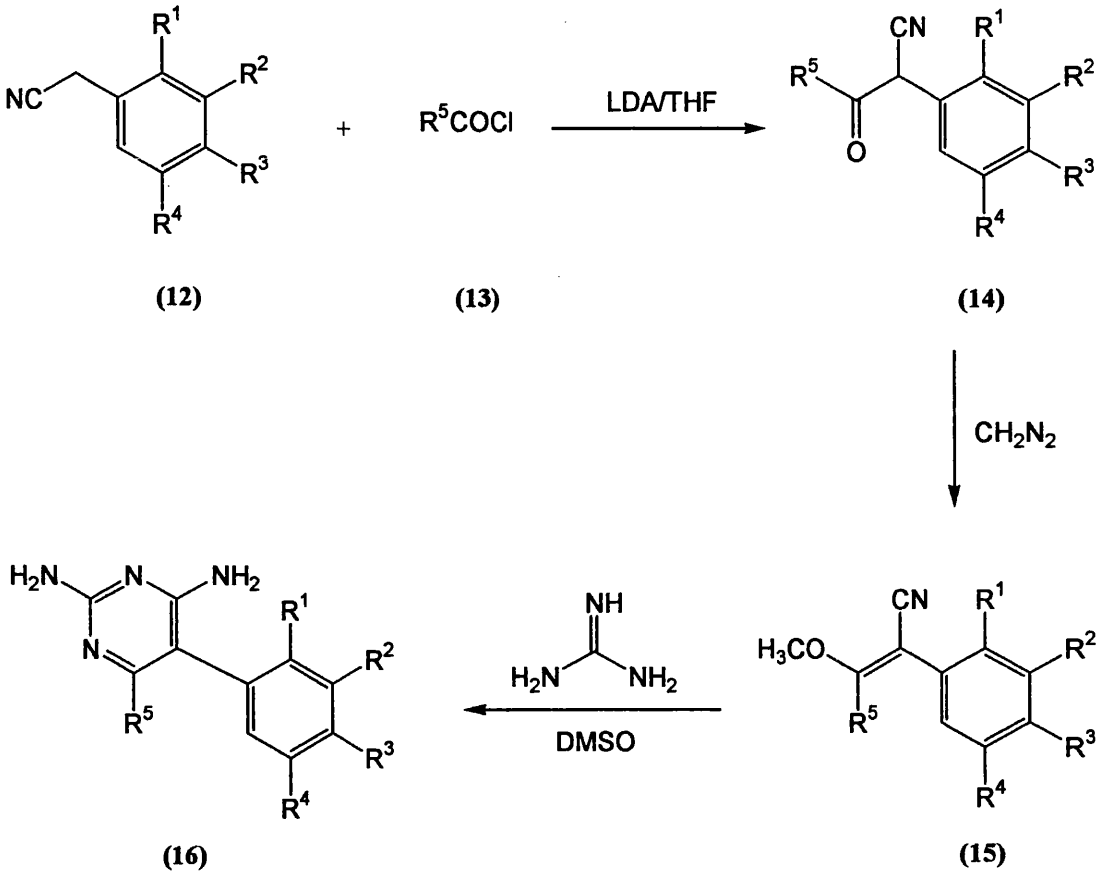



**(10)** **(11)**

**Scheme (9) Preparation of 2-amino-5-phenylpyrimidine**

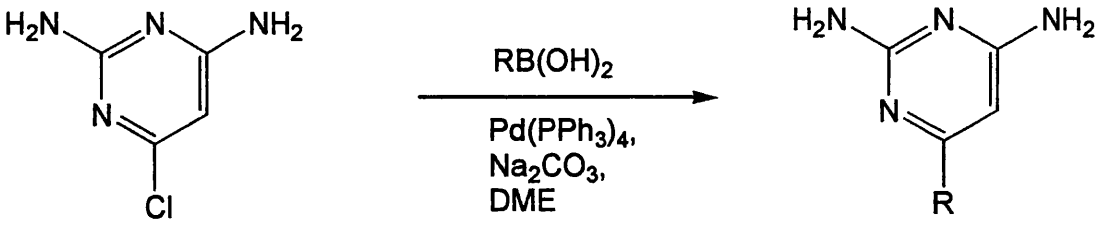
A modified method has been employed by Tarnchompoo and Sirichaiwat,[68] for the preparation of pyrimidine analogues. This improved synthetic route is outlined in (scheme 10). Lithiation of phenylacetonitrile derivatives (12) with lithium diisopropylamide (LDA) followed by acylation with acid chloride (13) afforded the corresponding acylphenylacetonitrile (14). Upon treatment of (14) with diazomethane, it provided the corresponding methoxyacrylonitrile (15), which was

subjected to the reaction with guanidine to furnish finally 2,4-diaminopyrimidine (16).




**Scheme (10) General procedure for synthesis of 2,4-diaminopyrimidine derivatives from phenylacetonitrile and acid chlorides**

Recently the Suzuki reaction, which involves the palladium-catalysed cross-coupling of heteroaryl-halides with arylboronic acids, has been used to prepare 6-aryl-2,4-diaminopyrimidines (18) from 6-halo-2,4-diaminopyrimidine (17) building blocks in relatively high yields (73-86%) (scheme 11).[116]

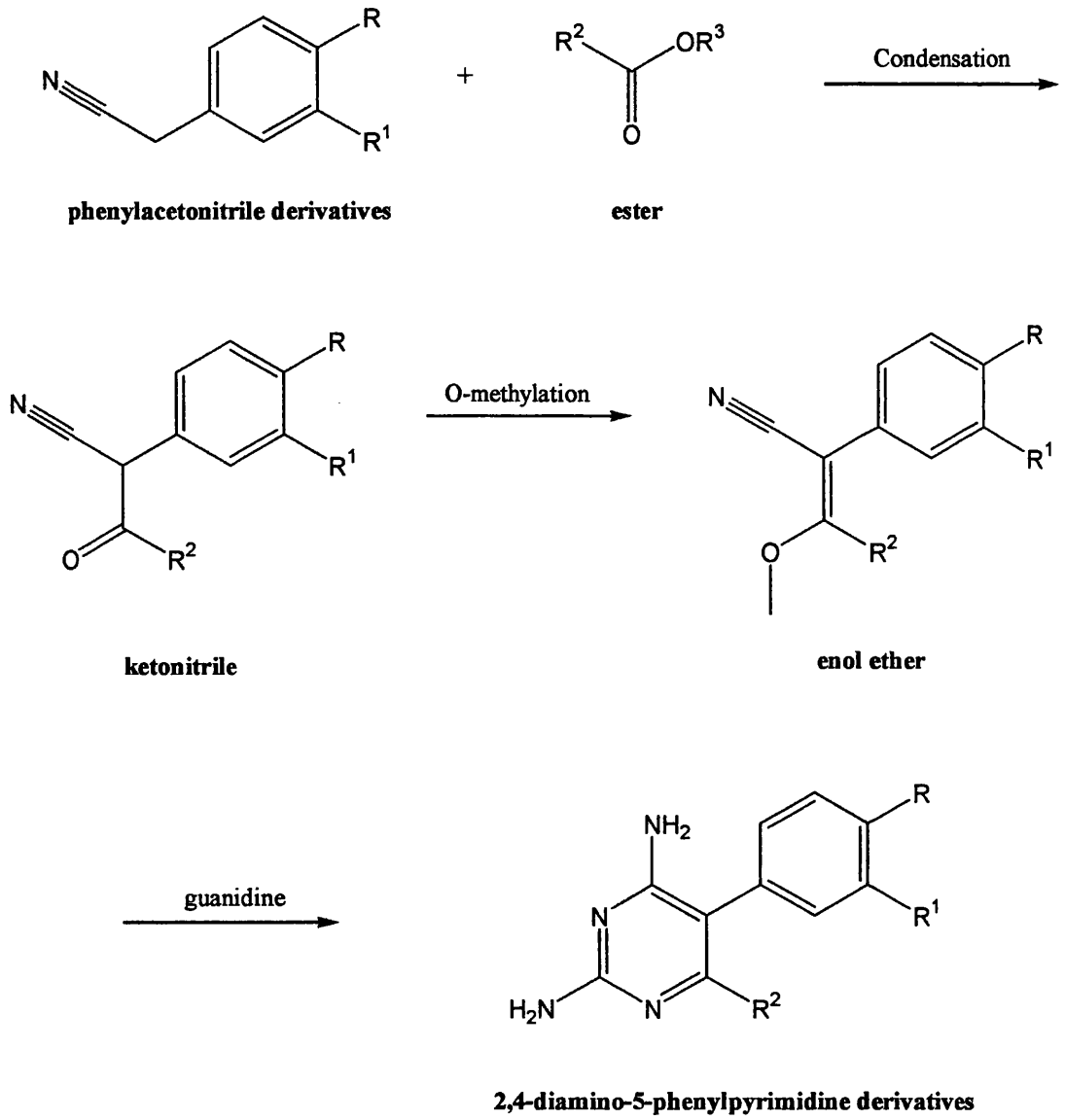



(17) (18)

**Scheme (11) Synthesis of 2,4-diamino-6-arylpyrimidines using Suzuki cross-coupling reactions**

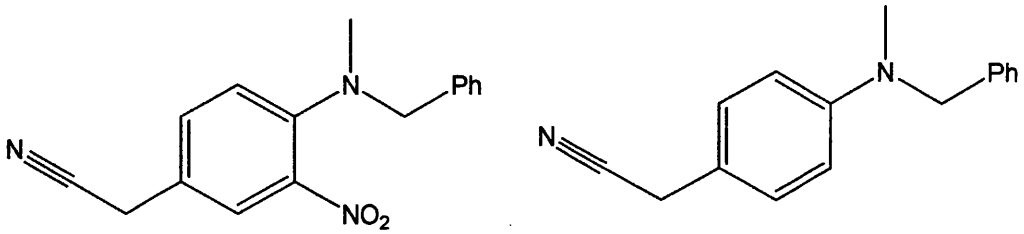
### 3.5 Strategy for Synthesis of the Target Compounds

The most convenient procedure that provides the mildest conditions that could be compatible with the trihydroxyalkyl side-chain at the 6-position of the target compounds is the strategy outlined in scheme (12). The key step for the synthesis of 2,4-diamino-5-(substituted phenyl)pyrimidine nucleus is the condensation of different derivatives of phenylacetonitrile with the appropriate esters to form the β-ketonitriles. The second step was the synthesis of the enol ethers through O-methylation of the β-ketonitriles. The final step would be condensation of guanidine with the different enol ethers to afford the target 2,4-diamino-5-(substituted phenyl)pyrimidines.




**Scheme (12) Strategy for synthesis of 2,4-diamino-5-(substituted phenyl)pyrimidine**

According to our strategy for the preparation of target groups of compounds, different phenylacetonitrile derivatives have been designed to be condensed with different esters to give the corresponding β-ketonitriles. Phenylacetonitriles with 4-chloro, 4-bromo and 3,4-dichloro substituents were commercially available. Phenylacetonitrile derivatives (19) and (20) were *novel* and have to be synthesized, (fig. 20).




(19) (20)

**Fig. (20) Structure of phenylacetonitrile derivatives**

The first series of target compounds, group A, contain a 3,4,5-trihydroxypentyl group linked to the 6-position of the pyrimidine ring (fig. 15). Two of these hydroxy groups are attached to two chiral carbons with (3*R*,4*S*) configuration.

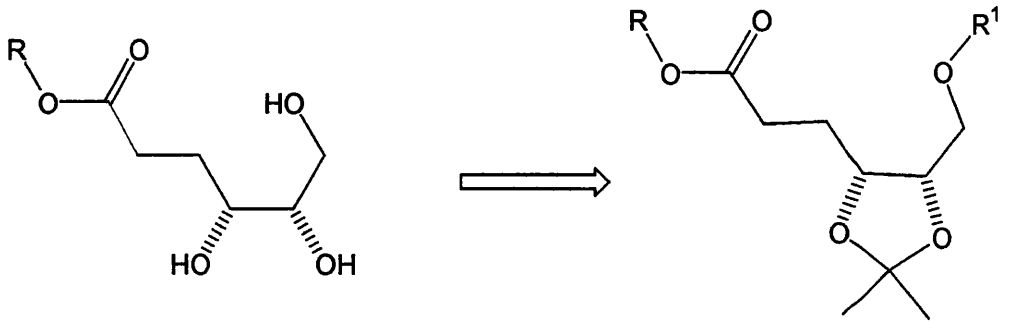
Retrosynthetic analysis of the pyrimidine synthesis revealed that this part of the structure derived from the corresponding ester (21) carrying three hydroxy groups (scheme 13), which was not commercially available. A common requirement in synthesis is that a hydroxy group should be masked as a derivative lacking a hydroxylic proton. Such a requirement appeared in the reaction of phenylacetonitrile with a strong base to generate the corresponding anion, which would react with ester to give the β-ketonitrile derivatives (scheme 12). Being aware of the acidic hydrogen of the hydroxy group (the $pK_a$ of $RCH_2OH$ = 16,[117] while $pK_a$ of $RCH_2CN$ = 25)[118] that could destroy one equivalent of the strong base used in the latter reaction and possibly adversely affect the reaction in other ways,[117] the retrosynthetic analysis of the structure of ester (21), scheme (13), revealed that these hydroxy groups should be protected with suitable protecting groups that keep the hydroxy groups protected along the synthetic pathway.

The most common route for the protection of vicinal hydroxy groups is formation of an acetal.[119] The third hydroxy group should be protected with another protecting group such as the formation of the benzyl ether as in ester (22). The acetal protecting group is inert to nucleophilic reagents and is unchanged under conditions such as hydride reduction, organometallic reactions or aqueous base-catalysed reactions. It also protects the hydroxy group against oxidation.[120,121] The benzyl ether

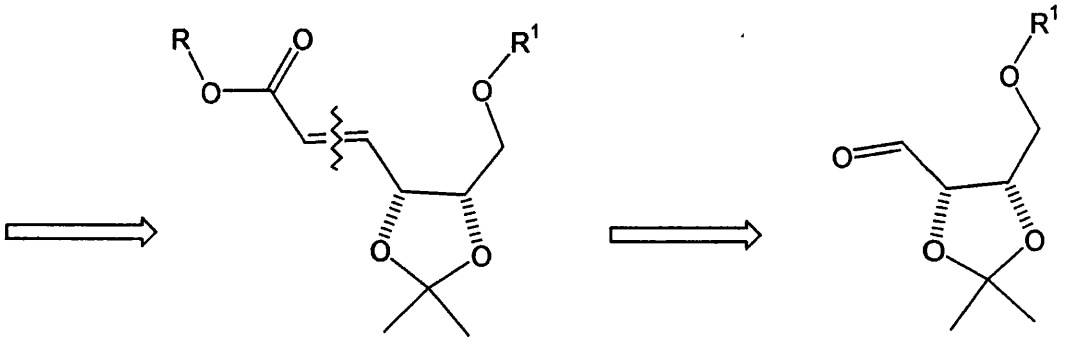
linkage is also providing protection to the primary hydroxy group towards nucleophilic reagents, oxidation reactions and aqueous base or acid-catalysed reactions.[122]

Consequently, we could proceed through the different reaction conditions involved in scheme (12) and scheme (13) while keeping the hydroxy groups unreactive towards all the reagents used along the synthetic pathway. The proposed ester (22) could be derived from the corresponding unsaturated ester (23) through Wittig reaction of the triphenylphosphoranylidene ester (24) with aldehyde (25). The latter could be derived from oxidation of the corresponding alcohol (26).

The second group of the target compounds (group B), contains the same functional group at 6-position of the pyrimidine ring apart from the configuration of one hydroxy group which had been manipulated to afford the corresponding (3*S*,4*S*)-3,4,5-trihydroxypentyl derivatives. The corresponding ester was not commercially available and should be synthesised using the same principle for the preparation of ester (21) in scheme (13), being aware of the change in configuration of the chiral carbons.

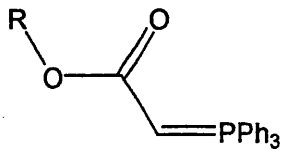


ester (21) ester (22)

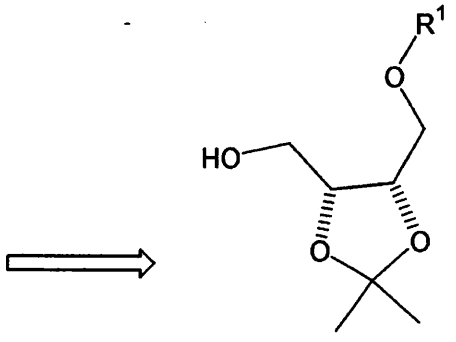


ester (23) aldehyde (25)

+



(24)

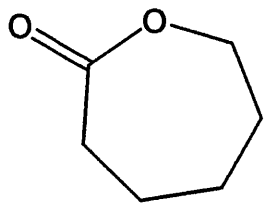


alcohol (26)

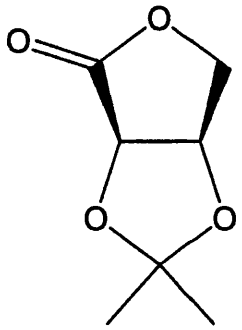
**Scheme (13) Retrosynthetic analysis of the 3,4,5-trihydroxyhexanoate ester**

Our strategy for preparation of group C of target compounds was to use the commercially available ε-caprolactone (27) (fig. 21), rather than the corresponding hydroxy ester, which is a *novel* method for preparation of β-ketonitrile.

The use of the corresponding lactone has the advantage of having intramolecular protection of the hydroxy group which in turn could shorten two steps along the synthetic pathway, one for protection and second for deprotection of the hydroxy group. Thus it should also improve the overall yield of the final product.



(27)



(28)

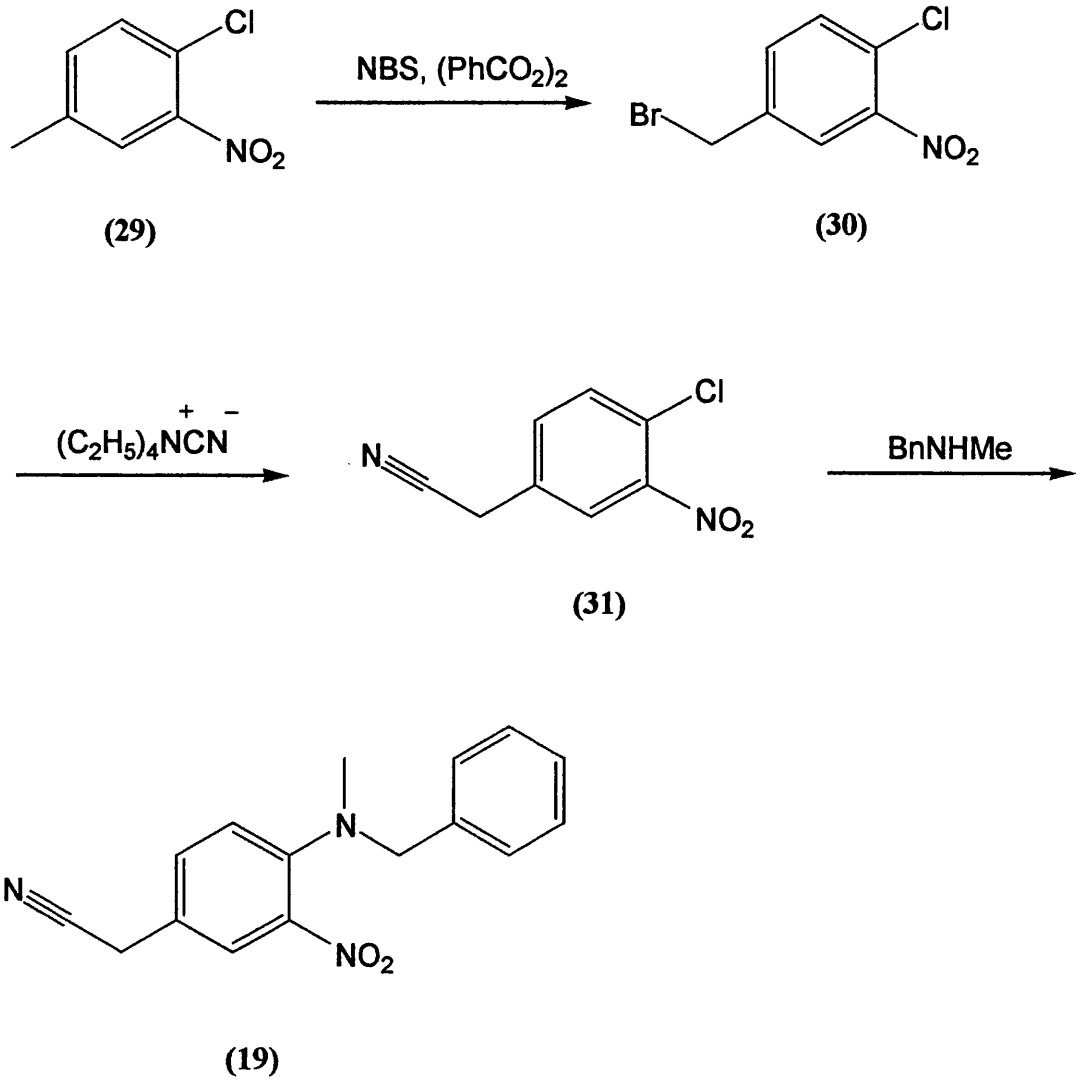
**Fig. (21) Structure of ε-caprolactone (27) and 2,3-O-isopropylidine-D-erythronolactone (28)**

Furthermore, we decided to prove the validity of the previous principle on the preparation of target compounds group D by using the commercially available lactone (28) (fig. 21), with a shorter carbon skeleton and two protected hydroxy groups, for preparation of the corresponding β-ketonitrile.

For the synthesis of model compounds, the commercially available ethyl acetate, ethyl 3-phenylpropanoate and ethyl propanoate were used as the ester counterpart for the synthesis of the corresponding β-ketonitriles.

### 3.6 Phenylacetonitrile and Its Derivatives

Phenylacetonitrile and its derivatives were used for condensation with esters or lactones to afford the corresponding β-ketonitrile. The *novel* 4-(N-benzyl-N-methylamino)-3-nitrophenylacetonitrile (19) was synthesised through radical monobromination of 4-chloro-3-nitrotoluene (29) using N-bromosuccinimide (NBS) and dibenzoyl peroxide as a radical initiator to give (30) in a good yield.[123]




**Scheme (14) Synthesis of 4-(N-benzyl-N-methylamino)-3-nitrophenylacetonitrile**

Compound (30) has two different halogens which are susceptible to nucleophilic substitution with a strong nucleophile such as cyanide ion ($CN^-$), with two different mechanisms.

The first one is through the bimolecular nucleophilic substitution ($S_N2$ mechanism) of the bromide on the aliphatic side-chain where the nucleophile approaches the substrate from a position 180° away from the leaving group (Br). The reaction is a one-step process with no intermediate. The identity of the leaving group influences the rate of $S_N2$ reaction because it departs with the pair of electrons from the covalent bond to the reacting carbon atom. The leaving group comes off more easily the more stable it is as a free entity. A correlation with electronegativity may be expected. The order of the halide as a good leaving groups is I > Br > Cl > F. This order is opposite from that of electronegativity and is dominated by the strength of the bond to carbon which ranges from ~ 50 Kcal $mol^{-1}$ for C-I bond to ~ 100 Kcal $mol^{-1}$ for C-F bond.[119] Thus iodide is the best leaving group and fluoride is the poorest.[124]

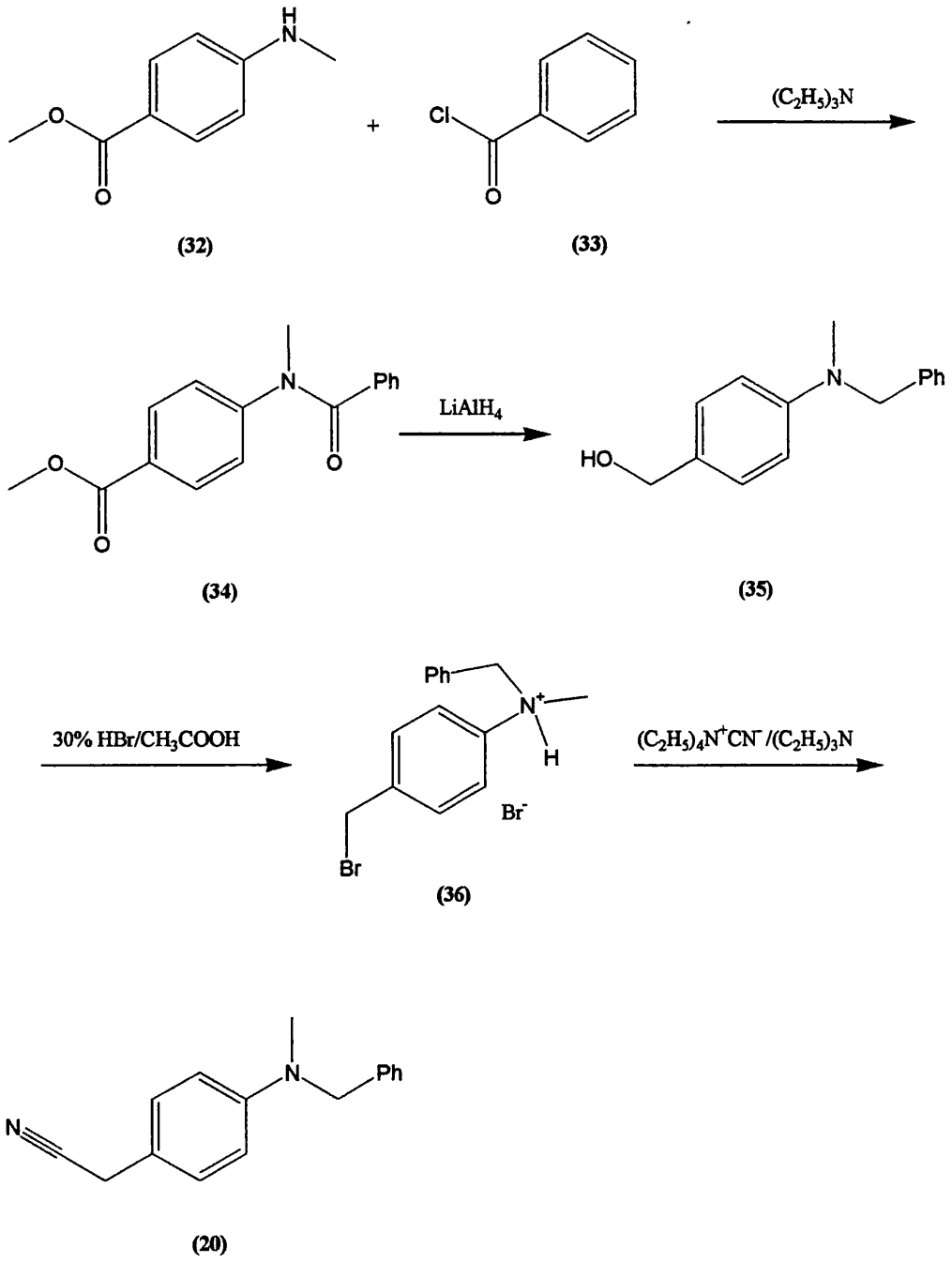
The second one is through aromatic nucleophilic substitution ($S_NAr$ mechanism) of the chloride on the benzene ring in two steps where the nucleophile forms a bond with the substrate first, giving a tetrahedral intermediate, (the rate-determining step) and then the leaving group (chloride) departs. An increase in electronegativity of the halogen as a leaving group causes a decrease in the electron density at the site of attack resulting in a faster attack by a nucleophile. Thus when fluoride is the leaving group, the relative rate was 3300 (compared with I = 1). Thus fluoride is the best leaving group while iodide is the poorest.[125]

The principal reason for the order I > Br > Cl > F in $S_N2$ reaction is the carbon-halogen bond strength which increases from I to F. The carbon-halogen bond strength is not so important a factor in $S_NAr$ because bond breaking is not ordinary part of the rate-determining step. Furthermore, the highly electronegative fluorine favours the addition step more than other halogens.[126] Consequently, by doing the nucleophilic substitution reaction under mild conditions, compound (30) was more active towards $S_N2$ mechanism than $S_NAr$ reaction as compound (30) reacted with

tetraethylammonium cyanide at room temperature to afford the nitrile derivative (31) but in a relatively low yield.[127]

The expected substituted phenylacetonitrile (19) was obtained (scheme 14) from the aromatic nucleophilic substitution ($S_NAr$) of the chloride in compound (31) with N-benzyl-N-methylamine under stronger conditions than the previous $S_N2$ reaction by heating under reflux overnight with the nucleophile.[128] The $S_NAr$ substitution reaction is accelerated by the electron–withdrawing effect of the nitro group *ortho* to the chloride because the addition step in the $S_NAr$ mechanism is greatly facilitated by strongly electron-withdrawing substituent, so that nitroaromatics are the best substrate for $S_NAr$ reactions.[125,129]

Another *novel* phenylacetonitrile derivative to be synthesised was 4-(N-benzyl-N-methylamino)phenylacetonitrile (20) (scheme 15). The first step was the formation of the amide derivative (34) by the acylation of methyl 4-(N-methylamino)benzoate (32) with benzoyl chloride (33) to afford the expected amide (34) in an excellent yield.[130] The reduction of (34) was established by using two equivalents of $LiAlH_4$ as a reducing agent in dry ether to afford the corresponding alcohol (35). Substitution of the hydroxy group by the bromide was obtained by the reaction of alcohol (35) with 30% hydrobromic acid in acetic acid to afford the hydrobromide salt (36).[131] Compound (36) was recovered in a very good yield as a very hygroscopic orange solid which could not be further purified. The $^1H$ NMR spectrum showed that it was sufficiently pure to be used as such for the following reaction. The corresponding free base of compound (36) was very unstable and could not be isolated by subsequent neutralisation with sodium bicarbonate. The presence of the bromide as a good leaving group along with such a strong nucleophile as the tertiary amine in one molecule makes it a very unstable one to be isolated, purified or even characterised.



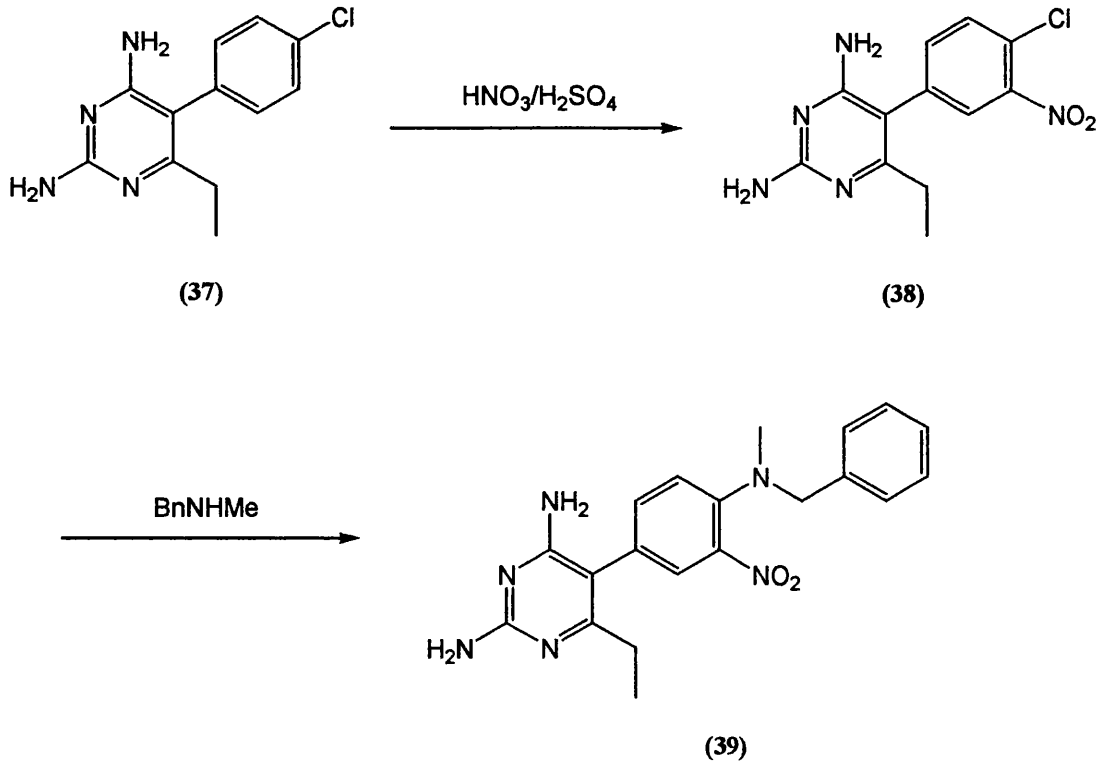

**Scheme (15) Synthesis of 4-(N-benzyl-N-methylamino)phenylacetonitrile**

The final step to target compound (20) was the nucleophilic substitution of the bromide with cyanide using tetraethylammonium cyanide as the nucleophile. The best reaction condition to get the required product (20) was by the addition of triethylamine first to liberate the free base followed immediately by the addition of

the nucleophile under dry conditions, and leaving the reaction mixture overnight to obtain the target compound (20).

Phenylacetonitriles (19) and (20) were prepared as starting phenylacetonitrile derivatives for the synthesis of the corresponding 2,4-diaminopyrimidine derivatives of the designed target compounds, as a similar 2,4-diaminopyrimidine derivative, methylbenzoprim (39), having the same side-chains on the phenyl group showed a strong inhibition of DHFR.[81,82]

In the literature, the preparation of methylbenzoprim (39) and its derivatives was derived from pyrimidine (37) (scheme 16). Compound (37) was nitrated in the *ortho* position to the chloride using $HNO_3/H_2SO_4$ mixture to give compound (38). The final step was then the $S_NAr$ nucleophilic substitution of the chloride with the secondary amine to give methylbenzoprim (39).[50,81,132]




(37) (38) (39)

**Scheme (16) Preparation of methylbenzoprim[50,81]**

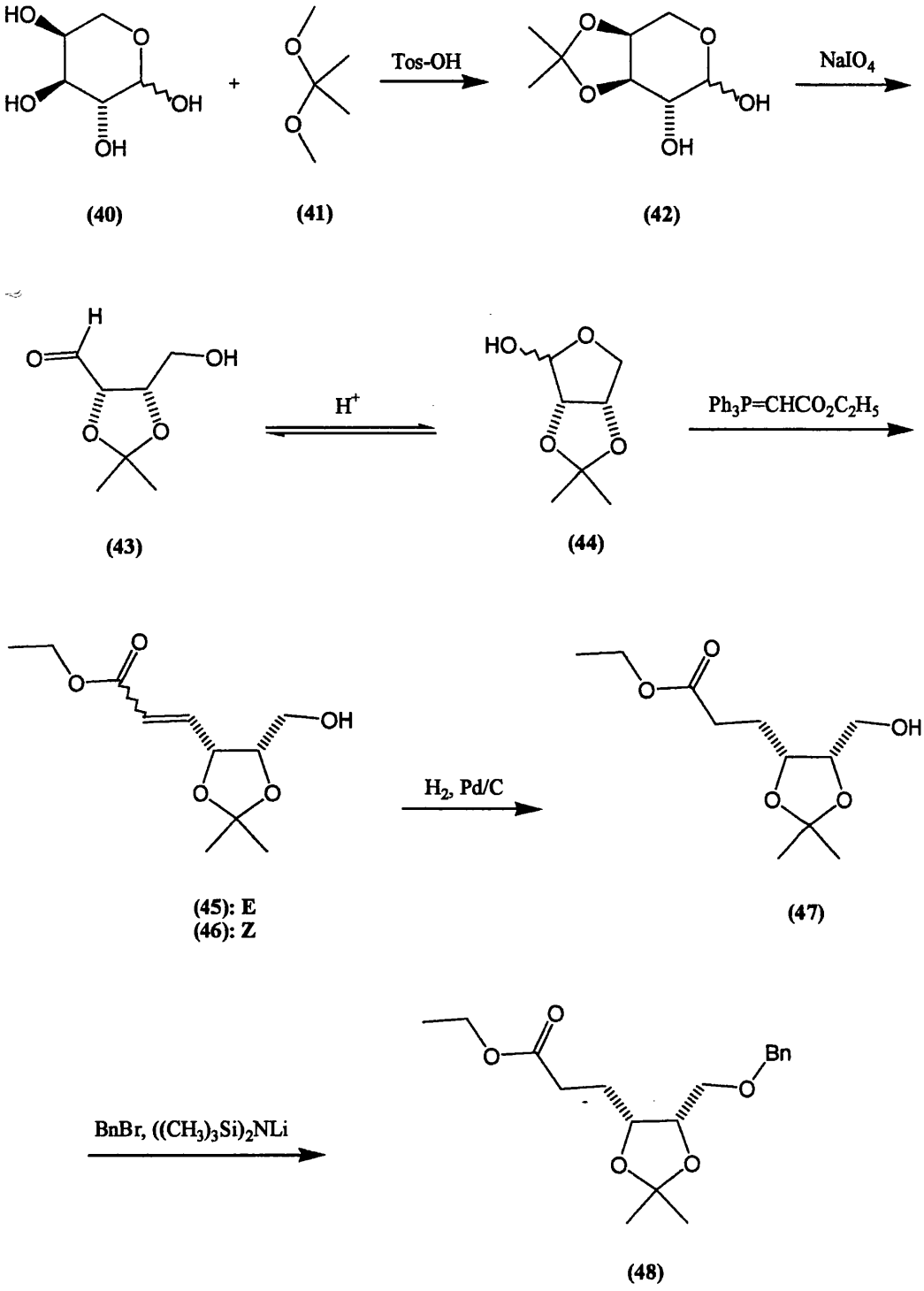
Our strategy was to incorporate these side-chains on the starting phenylacetonitrile derivatives as in compounds (19) and (20) before their condensation with the 3,4,5-trihydroxypentyl ester (22) to avoid the drastic condition of the nitration reaction which would destroy the polyhydroxylated side-chain at the 6-position of the target compounds.

### 3.7 Esters and Lactones

A variety of esters and lactones were used for condensation with different phenylacetonitriles for synthesis of the corresponding β-ketonitriles. For synthesis of group A target compounds, ester (48) (scheme 17) was to be synthesised for condensation with phenylacetonitrile and its derivatives to form the corresponding β-ketonitriles.

Retrosynthetically, ester (48) could be constructed from erythrose, by chain elongation at its terminus, enabling the eventual preparation of ester (48) as an enantiomerically pure substance. L-Arabinose (40) was the starting compound for preparation of erythrose (44),[133,134] according to scheme (17). Our strategy aimed to generate an aldehydic functional group at one particular side of the dioxolane ring followed by chain elongation at that end in order to end with the required configuration for ester (48).

Consequently the first step was protection of the vicinal hydroxy groups of L-arabinose (40) by reacting with 2,2-dimethoxypropane (41) to form the corresponding ketal (42) followed by periodate cleavage of compound (42) in one pot to give the aldehyde (43).[135,136] Ketal formation provide the required protection for the vicinal hydroxy groups against oxidation reactions, catalytic hydrogenation and nucleophilic substitution reactions as well as aqueous basic reactions. Formic acid, formed as a second product of the periodate oxidation of compound (42), catalyses the intramolecular cyclisation of the aldehyde (43) to the corresponding cyclic hemiacetal (44).[137]




**Scheme (17) Preparation of (4*S*,5*R*)-4-benzyloxymethyl-5-(ethoxycarbonylethyl)-2,2-dimethyl-1,3-dioxolane**

The reaction of aldehyde (43) with the Wittig reagent, ethyl triphenylphosphoranylidineacetate, afforded a mixture of (45) E and (46) Z alkenes, which were successfully separated by chromatography.[136,138] The Z geometry of the major product (46) (54% yield) was assigned by NMR $\delta_H$ spectroscopy. The signals due to the alkenyl protons (-CH=CH-) showed $J$ = 11.7 Hz. The corresponding signals in the E isomer (15) (15% yield) showed $J$ = 15.6 Hz. In addition, the 5-H signal (δ 5.65) in Z-isomer (16) appeared at considerably lower field compared with the corresponding 5-H in the E-isomer (15) (δ 4.79) due to deshielding by the ester carbonyl group.[139]

The reduction of a mixture of alkenes (45) and (46) was achieved by catalytic hydrogenation using 5% palladium on charcoal as a catalyst to afford (47) in a quantitative yield.[138] Protection of the hydroxy group of compound (47) through formation of benzyl ether was preferably carried out at the last step to avoid the cleavage of the ether linkage by the previous catalytic hydrogenation reaction. The final step was the protection of the hydroxy group of (47) to form the target ester (48) through the formation of the benzyl ether by reacting with benzyl bromide. The reaction proceeds by nucleophilic substitution of the bromide with alcohol (47) toward the formation of the ether linkage using lithium bis(trimethylsilyl)amide as a base to afford the target ester (48). Unfortunately, this reaction is only efficient on small scale (10 mmol or less). Working with larger scales resulted in formation of traces of the desired product. As a consequence, separate batches on small scales were run to prepare the required quantity of ester (48).

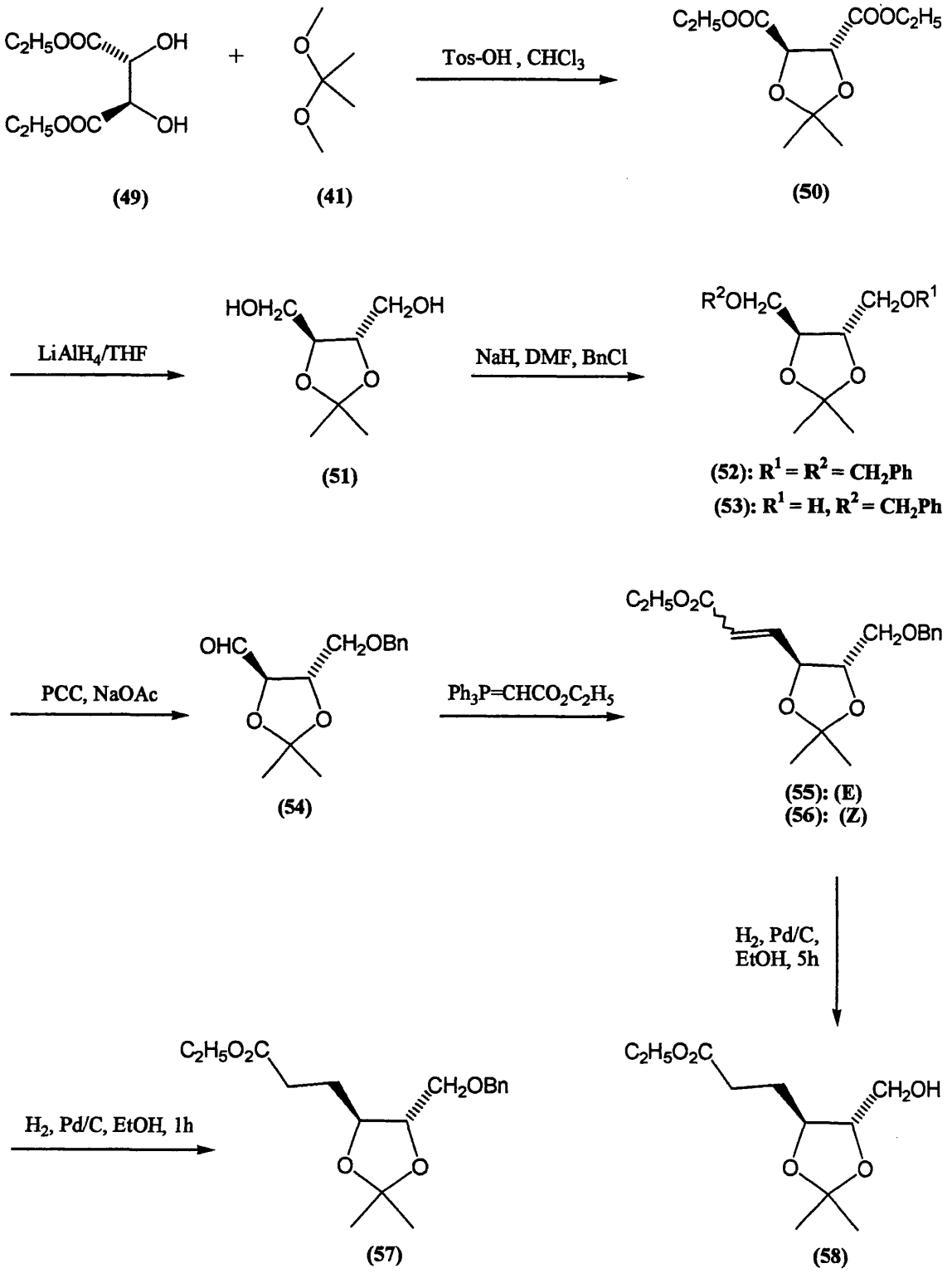
For synthesis of the group (B) target compounds, the ester counterpart required for coupling with phenylacetonitrile and its derivatives should be (4*S*,5S)-4-benzyloxymethyl-5-(ethoxycarbonylethyl)-2,2-dimethyl-1,3-dioxolane (57). The synthetic pathway employed for the preparation of compound (57) is outlined in scheme (18).

Our strategy for preparation of (57) was devised to construct the threo series 2,3-O-isopropylidenethreitol (50) and its derivatives (51-57) while keeping the hydroxy groups protected and the stereocentres enantiomerically pure. This approach was achieved by the protection of the vicinal hydroxy groups of diethyl *R*,*R*-2,3-

dihydroxybutanedioate (49) through ketal exchange with 2,2-dimethoxypropane (41) to afford the symmetrical ester (50) in a good yield.

Compound (50) was reduced to the corresponding $C_2$ symmetrical alcohol (51) in a good yield using $LiAlH_4$ as reducing agent.[140]

The $C_2$ symmetrical alcohol (51) has two equivalent alcoholic side-chains ($CH_2OH$) at C-4 and C-5; consequently protection of either of them and oxidation of the other will afford the same aldehyde. On the other hand, this strategy for preparation of ester (57) could not be used for preparation of its epimer (48) starting from the corresponding diethyl ester of *meso* tartaric acid as in such a case we would have a *meso* alcohol, the epimer of compound (51), with two non-equivalent side-chains at C-4 and C-5. Consequently, protection of one hydroxy group will result in formation of a pair of enantiomers which should be separated before proceeding to the oxidation step to ensure the enantiomeric purity of the target ester (48). Since enantiomers have identical physical properties and their separation is a complicated process so it was more convenient and practical to use a sugar as a starting material for the preparation of ester (48) as shown in scheme (17).




**Scheme (18) Preparation of (4*S*,5*S*)-4-benzyloxymethyl-5-(ethoxycarbonylethyl)-2,2-dimethyl-1,3-dioxolane**

The $C_2$ symmetry of alcohol (51) was then broken down by protection of one hydroxy group using a stoichiometric amount of benzyl chloride to give a mixture of compounds (52) and (53). Our investigation of the reaction conditions revealed that by using an equivalent amount of benzyl chloride and sodium hydride as a base and using dry DMF as a solvent, we have successfully prepared compound (53) in a good yield (64%), compared to compound (52) (28%), after an effective chromatographic separation.[141,142]

Pyridinium chlorochromate (PCC) is one of the reagents of choice for oxidation of primary alcohol to aldehyde[143,144] and was used for the oxidation of alcohol (53) to afford aldehyde (54) in a higher yield than that reported by Mukaiyama[145] by using Swern oxidation procedure using oxalyl chloride-activated dimethyl sulfoxide (DMSO) as oxidising agent. Furthermore, by using PCC, we avoided the use of excess reagent and formation of unpleasant-smelling volatile (boiling point 37 °C) by-product, dimethyl sulfide, on using Swern oxidation.[146,147]

Wittig reaction of aldehyde (54) with ethyl triphenylphosphoranylidineacetate afforded the adducts E (55) and Z (56) geometrical isomers, which were separated by column chromatography.[143] This reaction was achieved with unusual acid catalysis, as the presence of the carbonyl group in the phosphorus ylid resulting in decreasing its reactivity as a consequence of charge delocalisation which in turn reduces the nucleophilicity of the ylid.[123] Accordingly, acid-catalysis of the reaction should enhance the electrophilicity of the aldehyde and hence the reactivity of the aldehyde and as a result the reaction go to completion with a reasonably good yield.

The final step to the target ester (57) was the reduction of the side-chain double bond of a mixture of the unsaturated esters (55) and (56) without the cleavage of the benzyl ether linkage and, accordingly, deprotection of the primary hydroxy group. For that purpose, a carefully controlled catalytic hydrogenation of a mixture of esters (55) and (56) was investigated and the best reaction condition was achieved by using a catalytic amount of 5% palladium on charcoal and using ethanol as a solvent under an atmosphere of hydrogen for one hour to afford (57) in a good yield. Increasing the reaction time and the amount of the catalyst resulted in deprotection of the primary hydroxy group and accordingly would decrease the yield of the target

ester (57). Following the latter catalytic hydrogenation reaction by $^1$H NMR indicated that after two hours the cleavage of the ether linkage started as indicated in the $^1$H NMR spectrum by the disappearance of the characteristic peaks of the benzyl group protons and, after five hours, the benzyl group was completely removed and afforded compound (58).

The protection of alcohols as *tert*-butyldimethylsilyl (TBDMS) ethers has been recognised as one of the most useful methods because of its easy installation and general stability to basic, mild acidic reagents and hydrogenolysis but they are unstable to strong oxidising agents,[143,148,149] Consequently, silyl ethers could not be used for protection of the hydroxy group of the starting alcohol. The formation of a silyl ether is carried out by treatment of alcohol with the silyl chloride in the presence of a base.[149,150]

Silylation of alcohol (58) was tried by using either TBDMS chloride or *tert*-butyldiphenylsilyl (TBDPS) chloride with imidazole as a base and dimethylformamide (DMF) as solvent.[151,152] Such methods suffered from drawbacks such as the difficulty in removal of amine salts derived from the reaction of by-produced acid and co-bases during the silylation reaction,[150] and tedious and time consuming work up due to the presence of DMF which must be removed by repeated extraction of the mixture with water.[153] There was a visualization problem on following the reaction progress, in the case of using TBDMS chloride, by TLC as both the starting alcohol and the resulted ether are invisible under UV lamp or spraying with aqueous sulfuric acid. Consequently, purification by column chromatography was useless. Furthermore, silyl ethers are unstable under the PCC oxidation reaction condition as it is reported that PCC cleaved the TBDMS ether of *p*-hydroquinone at room temperature within 2 h.[143]

Consequently, it was then more convenient to reduce compound (56) to the target ester (57) and save protection and deprotection steps.

The prepared compounds were characterised by NMR spectrum which showed the characteristic peaks for each functional group transformation. The mass

spectrum and the accurate mass for the final ester (57) were also recorded. The specific rotations were also determined for the optically active compounds.

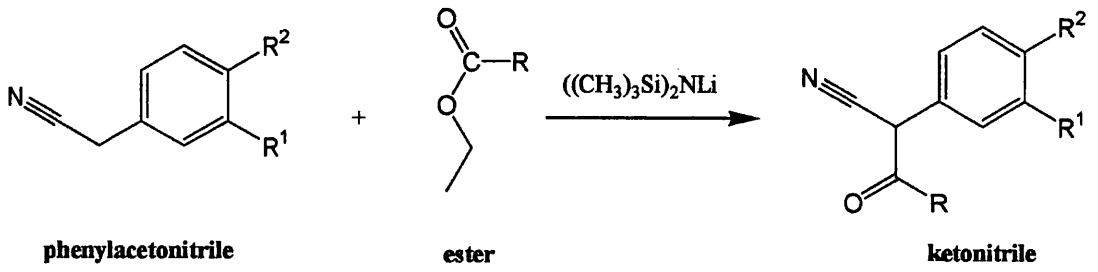
There are significant differences between the $^1$H NMR spectra of ester (48) and its epimer (57). The two methyl groups at C-2 of the dioxolane ring for ester (48) gave two singlets at δ 1.33 and δ 1.42 while the corresponding signals for ester (57) appeared at δ 1.38 and δ 1.39 as a result of changing the configuration of C-5. There are also significant differences in chemical shifts of the protons of the C-5 side chain between the two diastereoisomers. There is also a difference between signals due to the 4-H and 5-H on both diastereoisomers as they are present in different magnetic environment. In compound (48), the 5-H signal appeared as a multiplet overlapping with the quartet of the ethyl group at δ 4.08-4.15, while the 4-H with *cis* geometry to 5-H appeared as a double doublet at δ 4.28. The corresponding signals of compound (57) appeared as a multiplet for both 4-H and 5-H upfield at δ 3.80-3.87. These significant differences in $^1$H NMR indicated that we have successfully synthesised two different diastereoisomers during the two different synthetic pathways.

In addition to NMR characterisation, esters (48) and (57) have different optical activity values as ester (48) has $[\alpha]_D^{20}$ = +24.8° (c 4.4, $CHCl_3$) while ester (57) has $[\alpha]_D^{20}$ = -15° (c 4.0, $CHCl_3$) which indicate that they are diastereoisomers and not a pair of enantiomers.

A variety of commercially available esters and lactones has also been used for preparation of target groups C, D and model compounds. For synthesis of group C and group D target compounds, ε-caprolactone and 2,3-O-isopropylidene-D-erythronolactone, respectively, were used as they have the advantage of having the terminal hydroxy group intramolecularly protected compared with the open-chain ester analogues. Ethyl acetate, ethyl propanoate and ethyl 3-phenylpropanoate were successfully used as the starting ester counterparts for condensation with the phenylacetonitriles in preparation of β-ketonitriles to synthesis the model group of target compounds.

## 3.8 Preparation of β-Ketonitrile Derivatives

The first step for the preparation of our target compounds was the acylation of the phenylacetonitriles with different esters and lactones to form the corresponding β-ketonitriles (scheme 19).




**phenylacetonitrile** **ester** **ketonitrile**

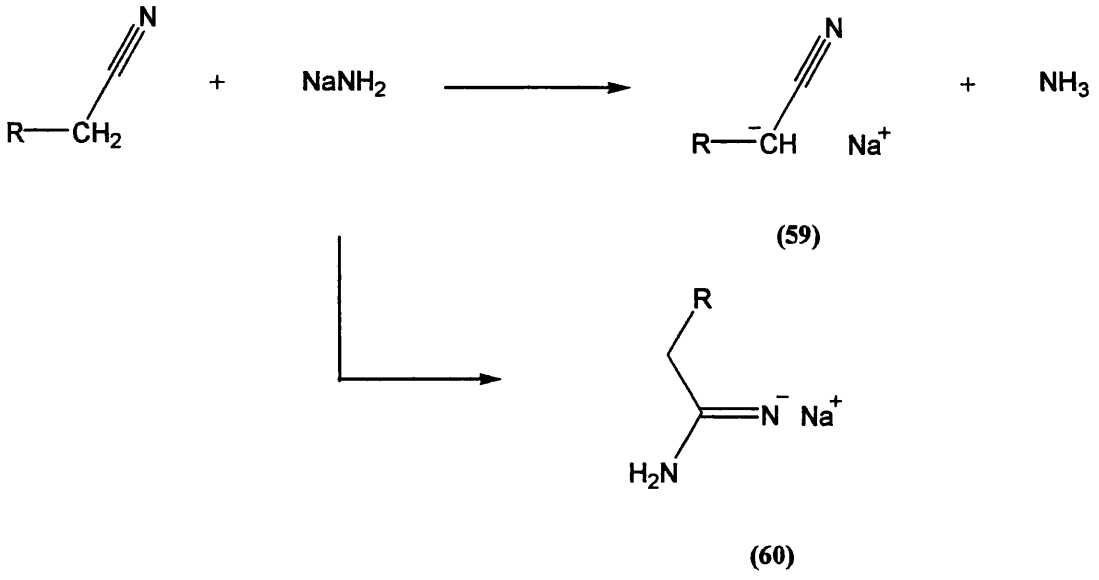
**Scheme (19) Preparation of β-ketonitrile derivatives**

The acylation of phenylacetonitrile with ethyl acetate was usually achieved by using sodium methoxide as a base[154,155] but, unfortunately, this method failed in the case of other esters or lactones. In the literature, Das and Boykin used sodium ethoxide as a base for acylation of phenylacetonitrile with ethyl formate; however, in the case of acylation with ethyl propanoate, the yield was very low, so alternatively they employed aprotic conditions and used sodium hydride (NaH) as the base.[156]

Bliss and Griffin also used sodium ethoxide as a base for the condensation of phenylacetonitriles with ethyl acetate or ethyl propanoate; however, problems were encountered at the condensation of 2-chlorophenylacetonitrile with ethyl propanoate as only (10% yield) of the corresponding β-ketonitrile was formed under optimum conditions. Furthermore, 2-methoxyphenylacetonitrile did not condense with ethyl propanoate, even under forcing conditions.[132]

Cook and Corly used the sodium salt of dimethylsulfoxide ($Na^+ {}^-CH_2SOCH_3$) as a base for acylation of phenylacetonitrile with esters to prepare the corresponding β-ketonitriles.[157] In a previous investigation a method was developed for acylation of nitriles with esters with sodium amide ($NaNH_2$) as a base but nitriles may be attacked

by sodium amide not only at α-hydrogen to form the sodionitrile (59) but also at the nitrile group to produce the sodium salt of the corresponding amidine (60) (scheme 20).[158,159]




**Scheme (20) Reaction of nitriles with sodium amide**

Such nitriles having only one α-hydrogen have been alkylated but special conditions (involving addition of sodium amide to a mixture of the nitrile and alkyl halide) appear to be required. Apparently some ionisation of α-hydrogen of these nitriles first occurs but the reaction gradually reverses in favor of amidine formation.[158]

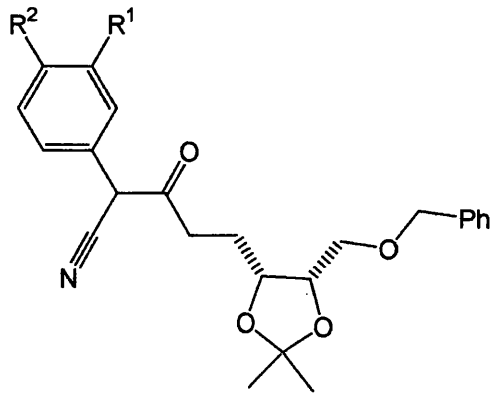
According to the Kayaleh and Gupta procedure, β-ketonitriles were produced from a Claisen condensation of benzoate esters with alkyl- or phenyl-acetonitriles in the presence of an excess of lithium diisopropylamide (LDA) as a base (4-6 equivalents). However, the yields were poor to modest, due to preferential attack of LDA (which is usually considered as non-nucleophilic) on the benzoate ester to give the N,N-diisopropylamide. Generally ethyl esters were used because they gave less of the N,N-diisopropylamide by-product than methyl esters. In no case was the reaction complicated by lithiation of the aryl ring derived from the acetonitrile even when methoxy groups were present.[160]

In the present work, many trials were made to achieve this condensation reaction by using a large variety of reported bases such as sodium alkoxides, sodium hydride and the sodium salt of dimethylsulfoxide under different conditions but they were all unsuccessful.

The possible complications with this condensation reaction were considered and rationalised. Firstly, the relatively high $pK_a$ values of nitriles (phenylacetonitrile $pK_a = 21.9$)[161] demand the utilisation of a strong base to abstract the α-hydrogen to form the corresponding anion.[162] Secondly, the acylation of nitriles furnishes a product (β-ketonitrile) which is considerably more acidic than the starting material.[163] Consequently, a strong, non-nucleophilic and sterically hindered base is needed to be able to abstract the proton from the nitriles and could not react with the product due to steric hindrance. Furthermore, the reaction condition was adjusted in such a procedure that prevents the Claisen condensation of two molecules of the esters together and formation of the β-keto ester,[112] rather than the target β-ketonitrile. Thus our strategy was to use an equivalent amount of the base to be added to the starting nitrile derivatives first to ensure the formation of the corresponding anion intermediate and at the same time ensure the complete consumption of the added amount of the base before the addition of the ester or lactone at low temperature (-78 °C) under dry conditions in order to minimise the possibility of Claisen condensation reaction and secure the formation of the target β-ketonitriles.[164]

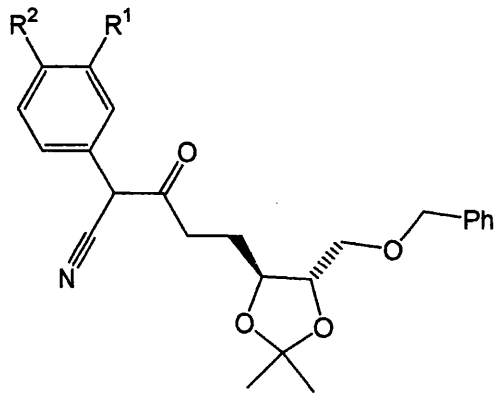
Lithium bis(trimethylsilyl)amide (LSA) was successfully used to favour the formation of the nitrile-anion to react with the ester or lactone as the electrophiles.[164] The advantage of using LSA as the base in this reaction is being a strong, non-nucleophilic base and being very bulky as well which, in turn, inhibits the nucleophilic addition of that base to the nitrile as in case of using sodium amide as a base which favour the formation of amidines.[159]

β-Ketonitriles (61-78) (fig. 22) were successfully synthesised and fully characterised by NMR spectroscopy.



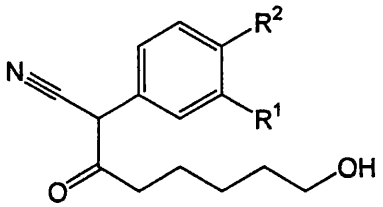
(61): $R^1 = H$, $R^2 = H$
(62): $R^1 = H$, $R^2 = Cl$
(63): $R^1 = H$, $R^2 = Br$
(64): $R^1 = Cl$, $R^2 = Cl$

The corresponding ketonitriles of group A



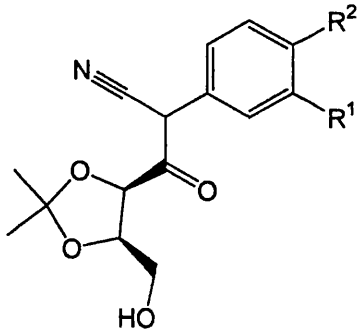
(65): $R^1 = H$, $R^2 = H$
(66): $R^1 = H$, $R^2 = Cl$
(67): $R^1 = H$, $R^2 = Br$

The corresponding ketonitriles of group B



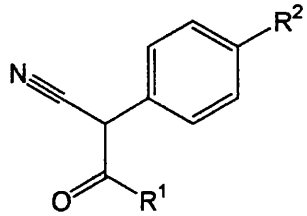
(68): $R^1 = H$, $R^2 = H$
(69): $R^1 = H$, $R^2 = Cl$
(70): $R^1 = H$, $R^2 = Br$
(71): $R^1 = Cl$, $R^2 = Cl$

The corresponding ketonitriles of group C



(72): $R^1 = H$, $R^2 = H$
(73): $R^1 = H$, $R^2 = Cl$
(74): $R^1 = H$, $R^2 = Br$
(75): $R^1 = Cl$, $R^2 = Cl$

The corresponding ketonitriles of group D



(76): $R^1 = CH_3$, $R^2 = H$
(77): $R^1 = CH_2CH_2Ph$, $R^2 = H$
(78): $R^1 = C_2H_5$, $R^2 = Cl$

The corresponding ketonitriles of model compounds

**Fig. (22) Structures of the prepared β-ketonitriles**

Assignment of $^1$H NMR spectral data of β-ketonitriles corresponding to group A and B target compounds revealed significant differences between the resonance and multiplicity of the protons of the aliphatic side-chain as a consequences of changing the configuration of one chiral centre. The 4-H on the dioxolane ring of group A compounds (61-64) resonated at the range of δ 3.90 to δ 4.29 while the corresponding proton of group B (65-67) resonated further upfield at the range of δ 3.53 to δ 4.03. Furthermore, the 5-H on the dioxolane ring of group A compounds resonated at the range of δ 4.20 to δ 4.22 as a quartet while the corresponding proton of group B compounds resonated further upfield at the range of δ 3.53 to δ 4.03 as a multiplet on its own or overlapping with the 4-H signals.

On the other hand, in case of group A compounds, the two protons of the ($CH_2OBn$) group resonated at the range of δ 3.45 to δ 3.49 while the corresponding protons of group B compounds resonated further downfield in the range of δ 3.55 to δ 3.89. Another feature was concerned with the protons of the $CH_2$ group attached to the chiral C-4 of the dioxolane ring. In the case of group A compounds, these protons resonated at the range of δ 1.66 to δ 1.88 as a multiplet with integration equivalent to two protons, while the corresponding protons of group B resonated further downfield at the range of δ 1.81 to δ 2.04 as they were appeared as a two sets of a well-separated multiplets.

These differences in the values of the chemical shift were because these protons are magnetically non-equivalent due to the presence of two chiral centres on the dioxolane ring. The inversion of configuration of C-5 in compounds of group B (65-67) was responsible for the previously mentioned differences of their $^1$H NMR spectra, compared to their epimers of group A compounds (61-64).

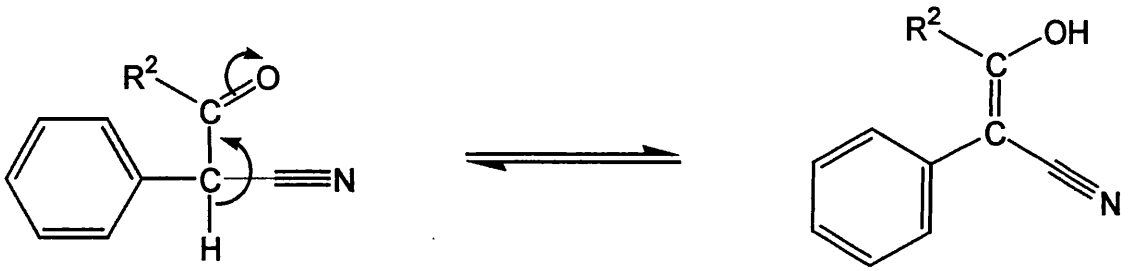
The assignment of the $^1$H NMR spectra of the aliphatic side chain of the β-ketonitriles (68-71) corresponding to group C target compounds called for doing the COSY spectra (COrrelated SpectroscopY), which indicated all the spin-spin coupled protons and hence enabled us to allocate the correct arrangement of the protons especially of the aliphatic side chain, on the NMR spectra in addition to the reported values of the chemical shifts of different groups. Methylene protons that are away

from the carbonyl and hydroxy groups should resonate upfield ($\delta_H$ = 1.4) followed by the methylene protons attached to the carbonyl group ($\delta_H$ = 2.9) and further downfield there should be the signals from the methylene protons attached to the hydroxy groups ($\delta_H$ = 3.6).[165]

The $^1$H NMR spectra of β-ketonitriles (68-71) of group C revealed that the 5-$H_2$ resonated relatively upfield at δ 1.21-1.42 as it was away from the deshielding effect of the hydroxy and carbonyl groups. According to the COSY spectra the latter protons were coupled with the 6-$H_2$ which resonated at δ 1.44-1.58. On the other hand, the former protons was reported also to be coupled with signals of 4-$H_2$ which resonated at δ 1.58-1.65. the latter protons were found to be coupled with 3-$H_2$ which resonated at δ 2.58-2.66 as a triplet for compound (68), or as two sets of douple triplet each integrating for one proton as in compounds (69 and 70), or as a multiplet as in compound (71). Finally, 7-$H_2$ were coupled to the 6-$H_2$ signals and resonated further downfield at δ 3.40-3.60 as a triplet due to the deshielding effect of the hydroxy group.

The $^1$H NMR spectra of β-ketonitriles of group D (72-75) were quite interesting. The methylene protons of the (C*H*$_2$OH) group are in a different magnetic environment because they are connected to a chiral atom (C-5) of the dioxolane ring and consequently resonated at different values. One of the latter protons resonated at δ 3.93-4.41 as a double doublet while the second proton resonated at δ 4.00-4.48 as a doublet due to geminal coupling with $J_{gem}$ = 10.1-11.0 Hz as recognised from the COSY spectra and values of the coupling constant.

The $^1$H NMR spectra of several of the prepared β-ketonitriles (61-78) revealed the presence of two tautomers, the enol and the keto-forms which is a very common form of tautomerism between a carbonyl compounds containing an α-hydrogen and its enol-form (scheme 21).




keto-form enol-form

**Scheme (21) Keto-enol tautomerism of β-ketonitrile**

In simple cases, the equilibrium lies well to the left as the keto form is thermodynamically more stable than the enol form.[166] However, in the case of β-ketonitriles (61-78), the enol form is the predominant form. The enol forms are more stable than the keto forms as the double bond of the enol form is in conjugation with the double bond system of the benzene ring in addition to the triple bond of the nitrile group. The enol forms are presumably the forms that are participating in the O-methylation reaction in a similar manner to the esterification of carboxylic acid.[167] The percentages of the enol-form and keto-form were determined from the integrations of the corresponding peaks to each form on the NMR spectra as provided in table (2) for the prepared β-ketonitriles (61-78).

| compound number | % of keto-form | Keto-form δ CHCN | % of enol-form | Enol-form δ OH | NMR $\delta_H$ solvent |
|---|---|---|---|---|---|
| 61 | 0 | _ | 100 | 8.96 | $CDCl_3$ |
| 62 | 10 | 5.52 | 90 | 9.33 | $CDCl_3$ |
| 63 | 35 | 5.48 | 65 | 9.35 | $CDCl_3$ |
| 64 | 0 | _ | 100 | 9.61 | $CDCl_3$ |
| 65 | 20 | 5.59 | 80 | 9.19 | $CDCl_3$ |
| 66 | 0 | _ | 100 | _ | $CDCl_3$ |
| 67 | 25 | 5.51 | 75 | 9.60 | $CDCl_3$ |
| 68 | 0 | _ | 100 | 11.52 | DMSO-$d_6$ |
| 69 | 100 | 4.65 | _ | _ | $CDCl_3$ |
| 70 | 100 | 4.65 | _ | _ | $CDCl_3$ |
| 71 | 100 | 4.69 | _ | _ | $CDCl_3$ |
| 72 | 0 | _ | 100 | _ | $CDCl_3$ |
| 73 | 0 | _ | 100 | _ | $CDCl_3$ |
| 74 | 35 | 5.57 | 75 | _ | $CDCl_3$ |
| 75 | 0 | _ | 100 | _ | $CDCl_3$ |
| 76 | 100 | 4.66 | _ | _ | $CDCl_3$ |
| 77 | 0 | _ | 100 | 11.70 | DMSO-$d_6$ |
| 78 | 0 | _ | 100 | 11.87 | $CDCl_3$ |

**Table (2) Percentage of keto and enol tautomers of the prepared β-ketonitriles**

Previous studies on keto-enol tautomerism of the diketo esters revealed that substituents with weak electronic demands may not have significant effect on the position of the equilibrium. On the other hand, strong electron-withdrawing *para* groups shift the equilibrium towards the keto-form.[168,169] This effect could be noticed in case of compounds of group A and B (61-67) as in case of compounds (62, 63 and 67), the *para*-chloro group in the phenyl ring slightly increases the percentage of the keto forms or has no effect as in compound (66) while the *para*-bromo substituent has a significant effect on the position of the equilibrium toward the formation of the keto form as observed in compounds (63 and 67).

The same observation was true for group D of β-ketonitriles (72-75). In the case of compounds of group C of the prepared β-ketonitriles (68-71), compounds (69-71) are present in the keto form. That may be due to the possibility of having intramolecular hydrogen-bond as well as intermolecular hydrogen-bond between the terminal primary hydroxy group (H-bond-donor) of one molecule and the carbonyl group (H-bond-acceptor) of another one in the keto-form make it more stable than the enol-form as long as the NMR spectra have been done in non-polar solvents such as $CDCl_3$.

The effect of the polarity of the solvent used on NMR on the keto-enol equilibrium is clearly apparent in the NMR spectrum of compound (68). A dramatic change on the equilibrium observed once using a polar solvent as DMSO-$d_6$ (H-bond acceptor) which favours the formation of the enol form (H-bond donor) and hence form hydrogen-bonds with both hydroxy groups rather than intramolecular or intermolecular ones.[170] Consequently, compound (68) is present as 100% enol form although the IR spectrum showed the characteristic peak of the carbonyl group at 1731 $cm^{-1}$.

Our early target was to prepare β-ketonitrile derivative (79) (scheme 22,1) by condensation of phenylacetonitrile (19) with an ester or lactone in order to increase the hydrophobicity of the corresponding 2,4-diaminopyrimidine derivative, as the Mtb-DHFR structure revealed the presence of pockets which are more hydrophobic in the mycobacterial enzyme than the human enzyme. That finding suggested that improved Mtb-DHFR inhibitor might be created by the addition of neutral and hydrophobic groups to these substituents would increase affinity and selectivity, and also beneficial in other ways, *e.g.* by enhancing the capability of compounds to pass the mycobacterial membrane.[88]

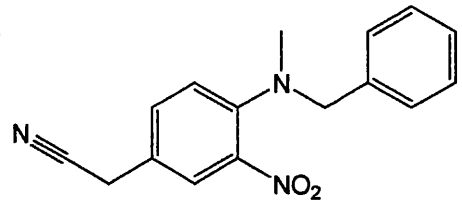
Considering compound (79), the presence of an electron-withdrawing (nitro group) would be predicted to reduce the basicity of the adjacent amine substituent and thus favour association with a hydrophobic domain at the active site of DHFR. Furthermore, our approach was to increase the reactivity to nucleophiles imparted upon the 4-chloro substituent by the introduction of a nitro group to displace the

chloro group with amine.[50] In the literature, the preparation of methylbenzoprim (MBP) and its derivatives (scheme 16) was started by reaction of 4-chlorophenylacetonitrile as the nitrile counterpart with an ester until complete synthesis of the corresponding pyrimethamine derivatives and followed by nitration *ortho* to the chloride. The final step was then the nucleophilic substitution of the chloride with the amino side chain.[50,81,132] In case of our target group of compounds (A-D) which contain one or more of the hydroxy group, final step nitration will be impractical as these hydroxy groups will not survive the drastic conditions of nitration reactions. Consequently, we decided to prepare the nitrated phenylacetonitrile derivative (19) as a starting material but unfortunately, its condensation reaction with different esters and lactones failed under different conditions, (scheme 22,1).

Another strategy was to synthesise phenylacetonitrile derivative (20) with an electron-donating group at the 4-position to activate the ring toward final step nitration of the corresponding 2,4-diaminopyrimidine derivative at 3-position under mild condition. We have successfully prepared compound (20) but unfortunately its condensation with any ester or lactone was unsuccessful, (scheme 22,2).

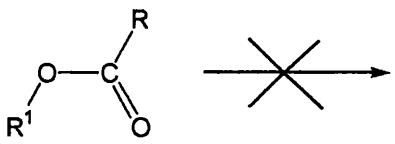
Finally, the condensation of 3,4-dichlorophenylacetonitrile with ester (57) failed to give the expected β-ketonitrile (81) (scheme 22,3), although it was successful with other esters and lactones.

1)-

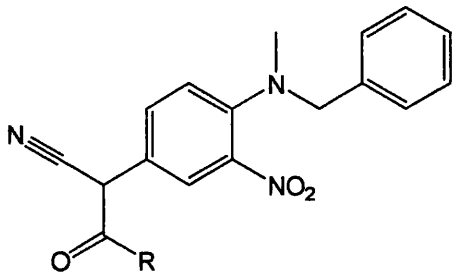


(19)

+

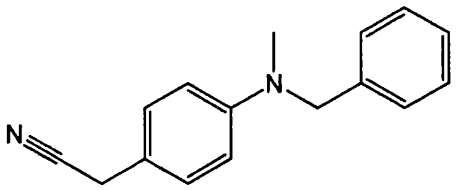


ester



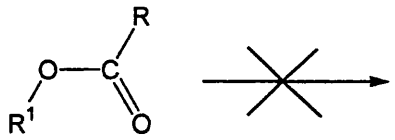
(79)

2)-

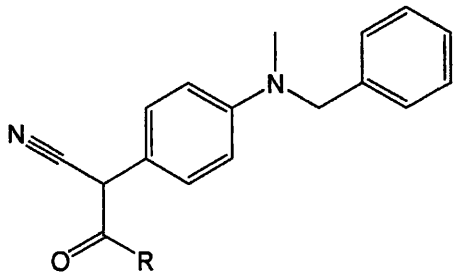


(20)

+



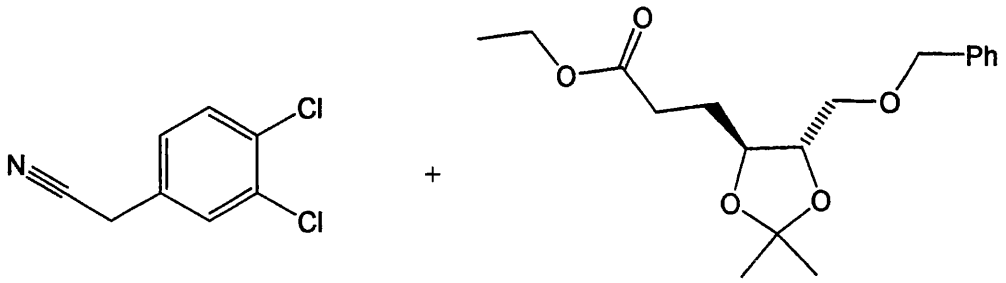
ester



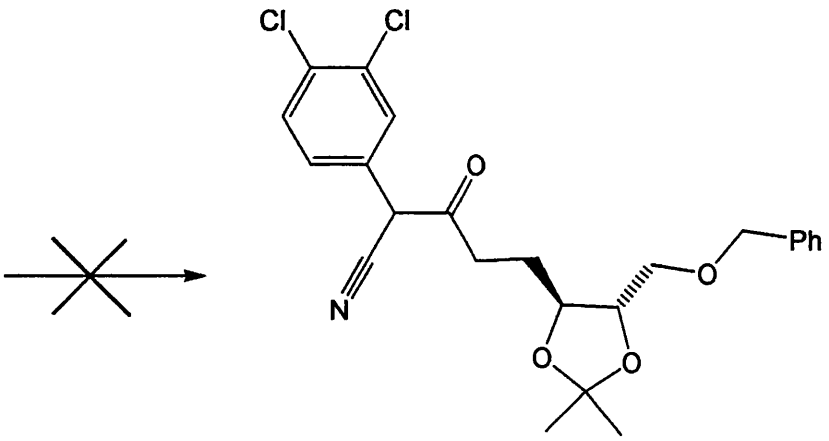
(80)

**Scheme (22) Unsuccessful trials for preparation of β-ketonitriles**

3)-



(57)



(81)

**Scheme (22) Unsuccessful trials for preparation of β-ketonitriles (continue)**

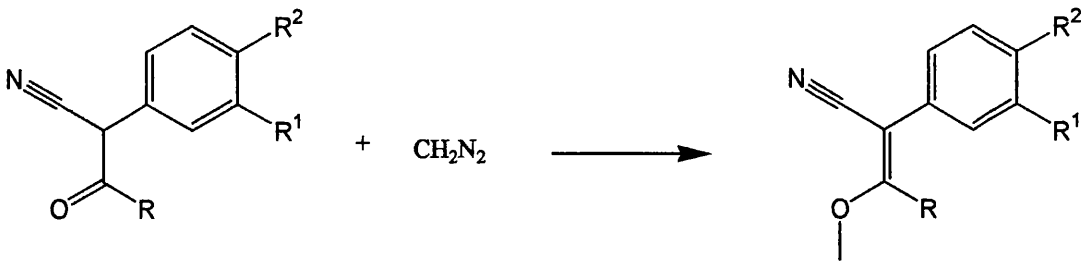
## 3.9 Preparation of Enol Ether Derivatives

Direct condensation of β-ketonitriles with guanidine was reported to form 2-amino-4-benzyl-1,3,5-triazines and thus led to the development of the enol ether sequence where enol ethers formed the desired pyrimidines and not benzyltriazines.[109,113]

The desired enol ethers were obtainable by treatment of β-ketonitriles with diazomethane. The products of the reaction of diazomethane with

α-acylphenylacetonitrile did in fact condense with guanidine to afford the 5-aryl-2,4-diaminopyrimidines in excellent yield.[113]

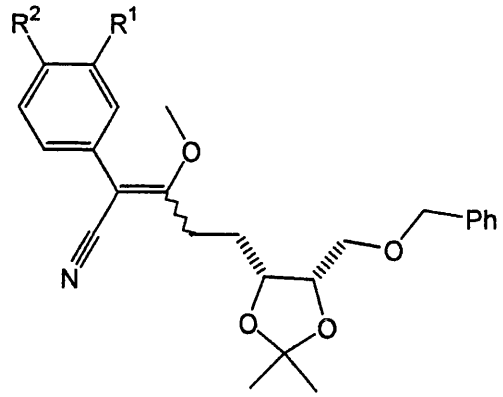
Having successfully synthesised the β-ketonitriles, preparation of the corresponding enol ethers (scheme 23) was then the next aim.




**ketonitrile** **diazomethane** **enol ether**

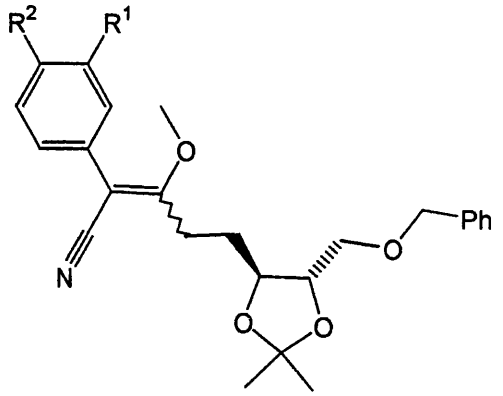
**Scheme (23) Preparation of enol ether derivatives**

O-Methylation of the β-ketonitriles (61-78) (fig.22) to afford the corresponding enol ethers (82-99) (fig. 23) was achieved successfully by using diazomethane ($CH_2N_2$), which is considered the most effective and common alkylating agent for carboxylic acids, phenols, enols and other compounds having acidic hydrogens and which are relatively hard nucleophiles.[171]



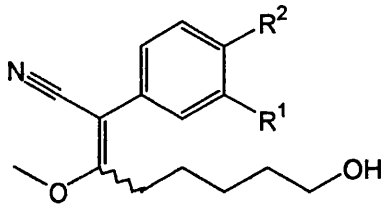
(82): $R^1 = H$, $R^2 = H$
(83): $R^1 = H$, $R^2 = Cl$
(84): $R^1 = H$, $R^2 = Br$
(85): $R^1 = Cl$, $R^2 = Cl$

The corresponding enol ethers of group A



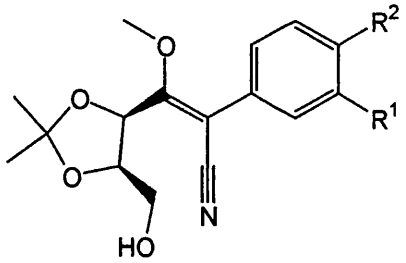
(86): $R^1 = H$, $R^2 = H$
(87): $R^1 = H$, $R^2 = Cl$
(88): $R^1 = H$, $R^2 = Br$

The corresponding enol ethers of group B



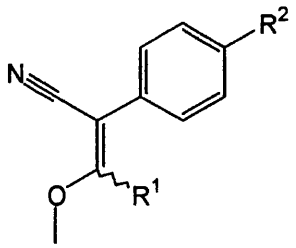
(89): $R^1 = H$, $R^2 = H$
(90): $R^1 = H$, $R^2 = Cl$
(91): $R^1 = H$, $R^2 = Br$
(92): $R^1 = Cl$, $R^2 = Cl$

The corresponding enol ethers of group C



(93): $R^1 = H$, $R^2 = H$
(94): $R^1 = H$, $R^2 = Cl$
(95): $R^1 = H$, $R^2 = Br$
(96): $R^1 = Cl$, $R^2 = Cl$

The corresponding enol ethers of group D

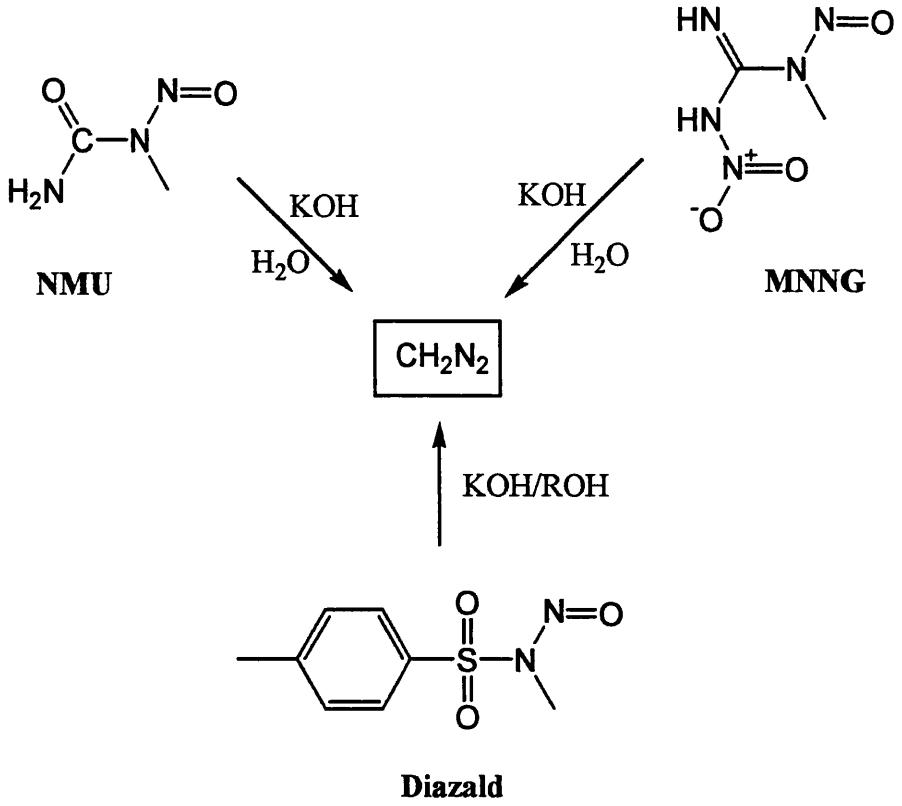


(97): $R^1 = CH_3$, $R^2 = H$
(98): $R^1 = CH_2CH_2Ph$, $R^2 = H$
(99): $R^1 = C_2H_5$, $R^2 = Cl$

The corresponding enol ethers of model compounds

**Fig. (23) Structures of the prepared enol ethers**

Diazomethane can be generated from different precursors such as N-methyl-N-nitrosourea (NMU),[172] N-methyl-N'-nitro-N-nitrosoguanidine (MNNG),[173] or N-methyl-N-nitroso-4-toluenesulfonamide (Diazald),[174] (scheme 24).




**Scheme (24) Preparation of diazomethane from different precursors**

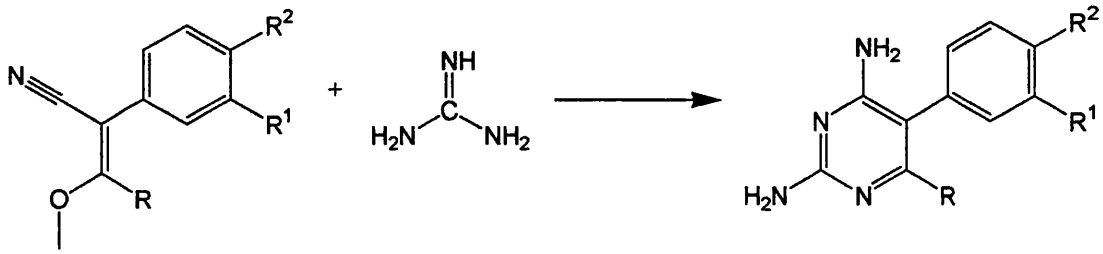
Diazald was used for generation of diazomethane by using the Aldrich Mini Diazald apparatus according to the Ngan and Toofan method.[171] Freshly synthesised diazomethane was used directly from the reaction apparatus for methylation of the β-ketonitriles to form the corresponding enol ethers (82-99) in very good yields.

The $^1$H NMR of the synthesised enol ethers revealed in some cases the presence of two geometrical isomers which could not be separated by chromatography or clearly identified by $^1$H NMR spectra. However, the overall structural assignments were corroborated by IR, mass and accurate mass spectroscopy.

On the other hand, whenever only one isomer was the predominant product, the $^1$H NMR spectra were completely clear and could be then resolved and recorded. Generally, the $^1$H NMR spectra of enol ether derivatives (48-65) revealed the presence of the characteristic peak of the protons of the methoxy group ($CH_3O$-) as a singlet in the range between δ 3.33 and δ 3.95.

### 3.10 Preparation of 2,4-Diaminopyrimidine Derivatives

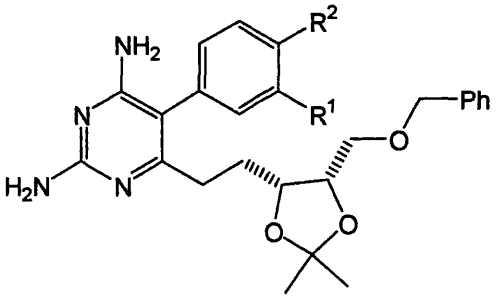
The target 2,4-diaminopyrimidine derivatives were prepared by cyclisation of the appropriate enol ethers derivatives (48-65) with guanidine (scheme 18), as it has been reported to be the most effective method for the preparation of 5-aryl derivatives of 2,4-diaminopyrimidine.[108,113,132]




**enol ether** **guanidine** **2,4-diaminopyrimidine**

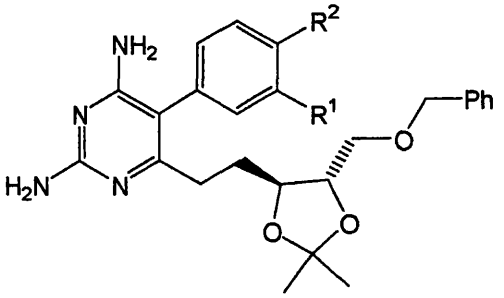
**Scheme (25) Preparation of 2,4-diaminopyrimidine derivatives**

The condensation of some enol ethers derivatives failed by using ethanol as a solvent but by replacing it by a higher boiling point alcohol such as 2-methoxyethanol (boiling point = 124 °C), the target pyrimidine derivatives (100-117) (fig. 24) were recovered faster and in good yields.



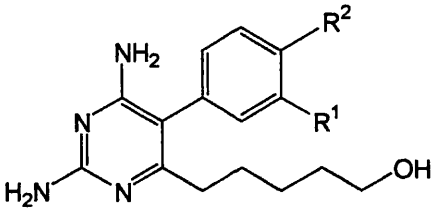
(100): $R^1 = H$, $R^2 = H$
(101): $R^1 = H$, $R^2 = Cl$
(102): $R^1 = H$, $R^2 = Br$
(103): $R^1 = Cl$, $R^2 = Cl$

corresponding 2,4-diaminopyrimidines (group A)



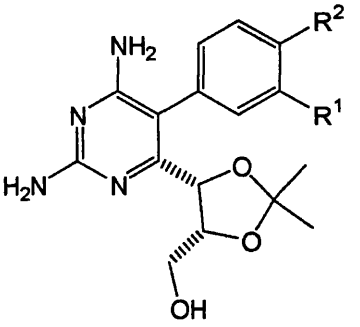
(104): $R^1 = H$, $R^2 = H$
(105): $R^1 = H$, $R^2 = Cl$
(106): $R^1 = H$, $R^2 = Br$

corresponding 2,4-diaminopyrimidines (group B)



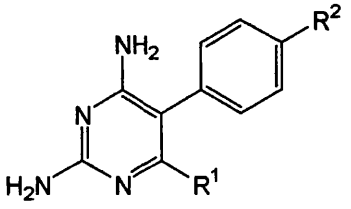
(107): $R^1 = H$, $R^2 = H$
(108): $R^1 = H$, $R^2 = Cl$
(109): $R^1 = H$, $R^2 = Br$
(110): $R^1 = Cl$, $R^2 = Cl$

corresponding 2,4-diaminopyrimidines (group C)



(111): $R^1 = H$, $R^2 = H$
(112): $R^1 = H$, $R^2 = Cl$
(113): $R^1 = H$, $R^2 = Br$
(114): $R^1 = Cl$, $R^2 = Cl$

corresponding 2,4-diaminopyrimidines (group D)



(115): $R^1 = CH_3$, $R^2 = H$
(116): $R^1 = CH_2CH_2Ph$, $R^2 = H$
(117): $R^1 = C_2H_5$, $R^2 = Cl$

corresponding 2,4-diaminopyrimidines (model compounds)

**Fig. (24) Structures of the prepared 2,4-diaminopyrimidine derivatives**

All of the *novel* products (100-114 and 116) were fully characterised by NMR spectroscopy as well as the mass and accurate mass spectra. In case of group (C) target compounds (107-110), and model compounds (115-117) (fig. 24), they are

the final products but, for others, there have been still further deprotection steps to liberate the hydroxy groups to afford the final target products.

Interestingly, the $^1$H NMR spectra of group D compounds (111-114) revealed that the 2- and 6-protons and the 3- and 5-protons of the benzene ring at the 5-position are diastereotopic in the cases of the H-compound, the monochloro- and the monobromo-compounds (111-113). The bulky group at the 6-position restricts the free rotation of the benzene ring in addition to the two chiral centres at the dioxolane ring as shown in (fig. 25), which was made by MM2 calculation energy minimisation, using Chem 3D 9.0 program. Consequently, these diastereotopic protons appeared at different chemical shifts and split each other. Furthermore, the two methyl groups of the dioxolane ring present in different magnetic environments and appeared as two well-separated singlets.

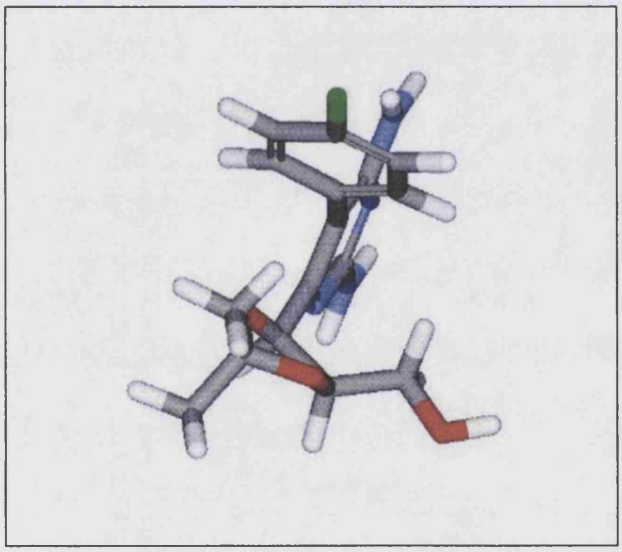


**Fig. (25) Stereoview of compound (112), carbon in grey, hydrogen in white, oxygen in red, nitrogen in blue and chloride in green**

In the $^1$H NMR spectrum of the monochloro-compound (112), these diastereotopic protons resonated at δ 7.05 for Ph 3-H, δ 7.27 for Ph 5-H, δ 7.43 for Ph 2-H and δ 7.47 for Ph 6-H. These protons split each other as each appeared as a doublet of doublets and the two methyl groups of the dioxolane ring appeared as two singlets at δ 1.24 and δ 1.63. On the contrary, the corresponding protons of the

benzene ring of the compounds of group B (104-106) are isochronous. In the $^{1}H$ NMR spectrum of compound (105), the protons of the benzene ring resonated at δ 7.10-7.35 as a multiplet because of the free rotation of the benzene ring and being away from the chiral centres of the dioxolane ring that make these protons magnetically equivalent. Furthermore, the two methyl groups of the dioxolane ring resonated at δ 1.28 and δ 1.31.

In the case of dichloro compound (114), there are two different conformers, as shown in (fig. 26), as a result of the restricted rotation of the benzene ring due to the bulky substituent at the 6-position. Thus the protons of the benzene ring of each conformer present in different magnetic environment because of the two chiral centres of the dioxolane ring at the 6-position.

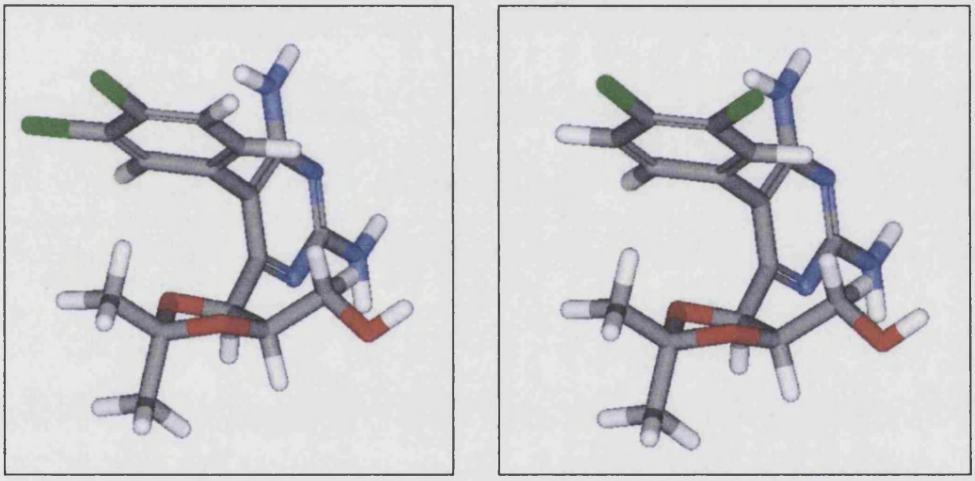


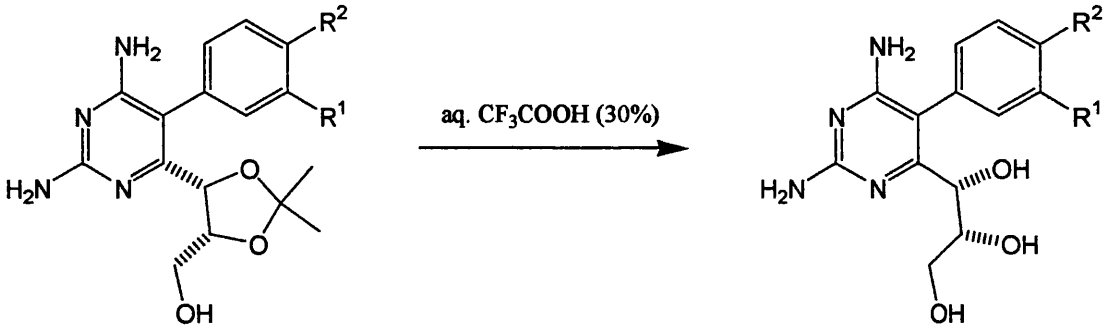
**Fig. (26) Stereoview of the two conformers for compound (114), carbon in grey, hydrogen in white, oxygen in red, nitrogen in blue and chloride in green**

In the $^{1}H$ NMR spectrum of compound (114), the signals of the protons of the benzene ring resonated at different values in 1:1 ratio. These protons for one conformer resonated at δ 7.11 for Ph 6-H as a doublet of doublets, δ 7.38 for Ph 2-H as a doublet and at δ 7.60 for Ph 5-H as a doublet. The corresponding protons of the other conformer resonated at δ 7.21 for Ph 6-H as a doublet of doublets, δ 7.48 for Ph 2-H as a doublet and at δ 7.62 for Ph 5-H as a doublet. Each of these peaks was

integrating for 0.5 protons. Furthermore, the two methyl groups of the dioxolane ring resonated at δ 1.20 and δ 1.49 as two singlets.

### 3.11 Deprotection of the Hydroxy Groups

Aiming to prepare the target compounds of groups A, B and D, deprotection of the hydroxy groups on the side chain was the last step for group D compounds (scheme 26).



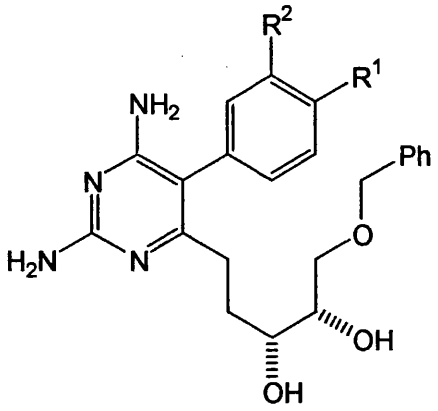

**protected compounds** **deprotected compounds**

**Scheme (26) Deprotection of group (D) compounds**

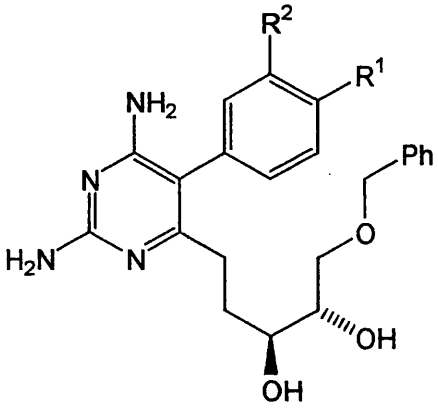
The hydroxy groups were protected from unwanted reactions until the last step because they are nucleophilic, weakly acidic ($pK_a$ 15-17), easily oxidised by a wide range of reagents and can participate in numerous transformations under mild conditions. Over 200 hydroxy protecting groups have been reported to date but, of these, only a comparatively small fraction are in common use,[122] such as the formation of silyl ethers,[175,176] alkyl ethers[177] and esters.[122]

Cyclic isopropylidene acetals (also known as acetonides) have been used more frequently than any other protecting group for the protection of 1,2- and 1,3-diols. The acetals are easily prepared and they are stable to most reaction conditions except protic and Lewis acids.[120] The isopropylidine acetals have been synthesised to protect the 1,2-diols in group A, B and D (fig. 24). Aqueous trifluoroacetic acid (30%)[178] was used successfully for cleavage of the dioxolane ring and deprotection

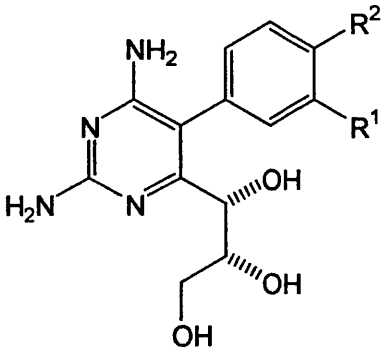
of 1,2-diol of compounds (100-106) and (111-114) to afford compounds (118-128) (fig. 27).




**(118): $R^1 = H$, $R^2 = H$**
**(119): $R^1 = H$, $R^2 = Cl$**
**(120): $R^1 = H$, $R^2 = Br$**
**(121): $R^1 = Cl$, $R^2 = Cl$**




**(122): $R^1 = H$, $R^2 = H$**
**(123): $R^1 = H$, $R^2 = Cl$**
**(124): $R^1 = H$, $R^2 = Br$**




**(125): $R^1 = H$, $R^2 = H$**
**(126): $R^1 = H$, $R^2 = Cl$**
**(127): $R^1 = H$, $R^2 = Br$**
**(128): $R^1 = Cl$, $R^2 = Cl$**

**Fig. (27) Structures of target compounds (118-128)**

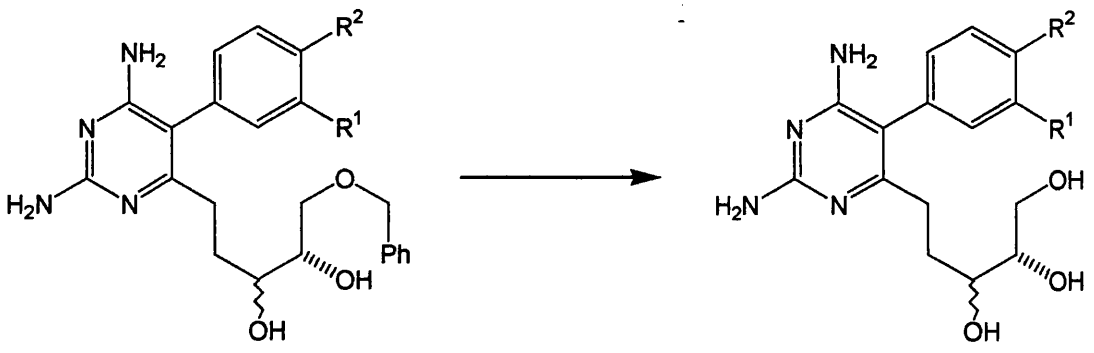
For group D target compounds, it was the last step and compounds (125-128) (fig. 27), are our final target *novel* products for group D, which have been completely characterised by NMR and mass spectra. The NMR spectra are characterised by the disappearance of the two upfield signals corresponding to the two methyl groups of the dioxolane ring as a result of the deprotection step.

The $^1H$ NMR spectra revealed that the (3-$H_2$) protons of the aliphatic side chain, because of being attached to the chiral (C-2) atom, resonated at δ 3.40-3.63 as two sets of double doublet each integrated for one proton for compounds (125-128) or as a multiplet for compound (128). The (2-H) proton resonated further downfield at δ 3.68-3.72 as a multiplet while the (1-H) proton resonated furthermore downfield at δ 4.35-4.54 as a douplet for compounds (125-128) because of the deshielding effect of the neighbouring oxygen and nitrogen.

In the $^{13}C$ NMR spectra and with the help of 135 DEPT spectra the (C-3) carbon was identified as the signal resonating at δ 62.60-63.67 as the corresponding peak projecting downwards in the DEPT spectra while the (C-1) and (C-2) carbons remain upwards and resonated further downfield at δ 68.98-74.29 for compounds (125-128).

The specific rotation $[\alpha]_D$ was also determined for compounds (125-128) as they rotated the plane of polarised light to the left and are designated (-), for example compound (126) has $[\alpha]^{20}_D = -41°$ (c 0.4, $CH_3OH$).

For group A and B target compounds the corresponding products (118-124) still need another step for cleavage of the benzyl ether protecting group (scheme 27) to afford the final target group A and B *novel* compounds.



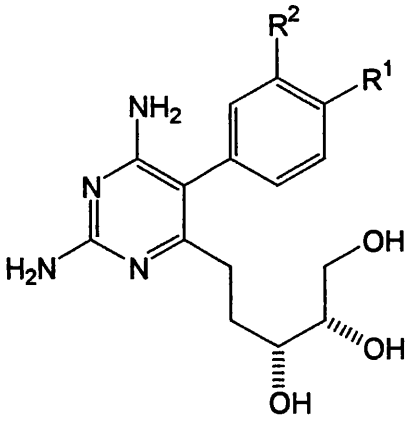

**protected groupA and B compounds** **target groupA and B compounds**

**Scheme (27) Deprotection of group A and B compounds**

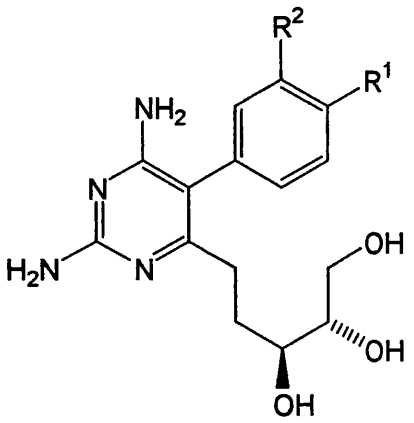
The cleavage of benzyl ether linkage of compounds (118-124) (fig. 27) was our last aim for preparation of my target compounds (129-135) (fig. 28).

Benzyl ethers are stable to a wide range of aqueous acidic and basic conditions. They are not readily attacked by most metal hydride reducing agents or mild oxidising agents.[122] Catalytic hydrogenolysis offers the mildest method for deprotecting benzyl ethers. The catalyst of choice is palladium on charcoal in THF, ethanol or ethyl acetate.[122,179]

Unfortunately, the latter method for deprotection was unsuccessful and the starting material was recovered after 96 hours. Addition of perchloric acid which often accelerates debenzylation, led to destruction of the compounds.




(129) : $R^1 = H$, $R^2 = H$
(130) : $R^1 = H$, $R^2 = Cl$
(131) : $R^1 = H$, $R^2 = Br$
(132): $R^1 = Cl$, $R^2 = Cl$




(133): $R^1 = H$, $R^2 = H$
(134): $R^1 = H$, $R^2 = Cl$
(135): $R^1 = H$, $R^2 = Br$

**Fig. (28) Structures of group A and B target compounds**

On the other hand, the addition of a few drops of chloroform to the catalytic hydrogenolysis deprotection procedure and using methanol as a solvent,[180] effectively deprotected compounds (118 and 122). However, in the cases of the deprotection of the 4-chloro and 4-bromo substituted compounds (119, 120, 123, 124) (fig. 27), dehalogenation took place in addition to the deprotection reaction as indicated by the NMR and mass spectra. The deprotection of compounds (119,120)

of group A by the latter method afforded the same product, compound (129), and it was also true for the corresponding compounds (123, 124) of group B. In the case of the 3,4-dichloro derivative (121) a mixture of chlorinated and dechlorinated derivatives was recovered and could not separated by chromatography due to the high polarity of the deprotected compounds.

Attempted removal of the benzyl group by dealkylation with HBr/HOAc resulted in a dramatic substitution of the hydroxy groups by bromides beside acetylation of the primary hydroxy group as well as formation of the hydrobromide salt of the pyrimidine ring.[181]

An alternative method for reductive deprotection was by using sodium metal and liquid ammonia[182] which successfully cleaved the benzyl ether for compounds (118 and 122). Unfortunately, the use of the latter method for deprotection of the halogenated compounds (119-124) resulted in dehalogenation of the benzene ring and consequently compounds (118-121) and compounds (122-124) afforded the dehalogenated products (129) and (133) respectively.

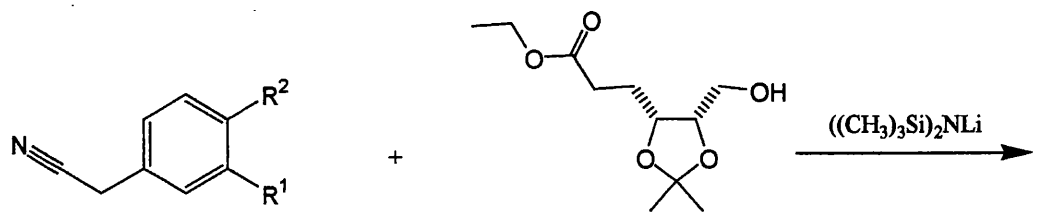
Although numerous methods are available for cleavage of benzyl ethers, problems arise in the presence of multiple functionalities. It was found that the use of anhydrous iron (III) chloride in dichloromethane is highly efficient for the cleavage of benzyl ethers at room temperature. The cleavage reactions presumably involve initial complexation of the Lewis acid iron (III) chloride to the ethereal oxygen atom and subsequent displacement with chloride to yield the free alcohols and benzyl chloride. This reaction does not proceed in protic or lone pair electron-rich solvents such as alcohols, diethyl ether and acetone.[183]

The use of anhydrous iron (III) chloride was effective and successfully debenzylated compounds (118-124) to the corresponding target compounds (129-135) without affecting the halogens on the benzene ring. Moreover, it was also a very effective method for deprotection of the isopropylidene protecting group of 1,2-diol of compounds (100-106 and 111-114) (fig. 24) as well. Following the deprotection of compounds (100-106 and 111-114) using the latter method by TLC and NMR, it was

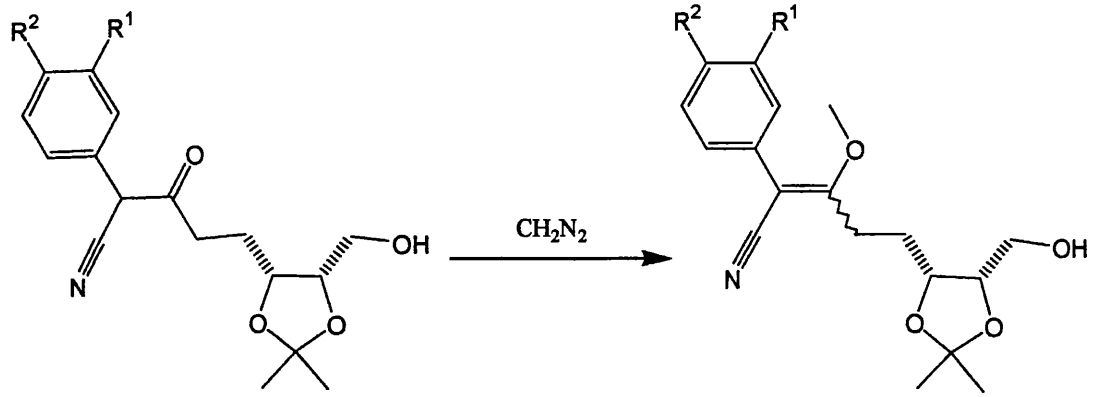
found that cleavage of the acetal linkage was achieved after 5 min and the debenzylation step was finished within 80 min.

Owing to the problems encountered with the final-step cleavage of the benzyl ether linkage and depending on our experience gained from the successful cyclisation of the enol ethers (89-96) (fig. 23) of the corresponding group C and D compounds without protecting the primary hydroxy group, We thought that it could be possible and worth trying to synthesise the corresponding β-ketonitriles for group A target compounds by condensation of the hydroxyl ester (47) with different derivatives of phenylacetonitrile to form the corresponding β-ketonitriles.

β-Ketonitrile derivatives (136-139) (scheme 28) were successfully prepared by the same method described before, which in turn O-methylated exclusively at the enol oxygen with diazomethane to afford the corresponding enol ethers (140-143). These were finally cyclised with guanidine to give the corresponding 2,4-diaminopyrimidine derivatives (144-147). By using aqueous trifluoroacetic acid (30%), we could effectively obtain the corresponding target group A compounds (129-132) (fig. 26). The spectroscopic analysis data for target group A compounds obtained by these two different starting esters were identical.




**phenylacetonitrile derivatives** **ester (47)**

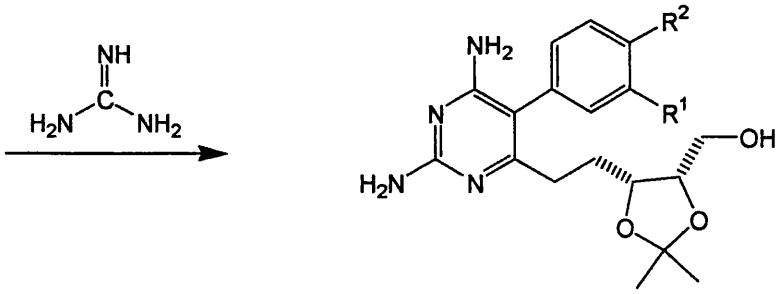



**(136): $R^1$ = H, $R^2$ = H**
**(137): $R^1$ = H, $R^2$ = Cl**
**(138): $R^1$ = H, $R^2$ = Br**
**(139): $R^1$ = Cl, $R^2$ = Cl**

The corresponding ketonitriles of group A

**(140): $R^1$ = H, $R^2$ = H**
**(141): $R^1$ = H, $R^2$ = Cl**
**(142): $R^1$ = H, $R^2$ = Br**
**(143): $R^1$ = Cl, $R^2$ = Cl**

The corresponding enol ethers of group A




**(144): $R^1$ = H, $R^2$ = H**
**(145): $R^1$ = H, $R^2$ = Cl**
**(146): $R^1$ = H, $R^2$ = Br**
**(147): $R^1$ = Cl, $R^2$ = Cl**

The corresponding 2,4-diaminopyrimidines of group A

**Scheme (28) Preparation of 2,4-diaminopyrimidine derivatives by using the hydroxy ester (47)**

The $^1$H NMR spectra of group A and B compounds (129-135) (fig. 26) have significant differences in chemical shifts of the protons of the aliphatic side chain carried on the two chiral carbons (C-3 and C-4). The (3-H) proton resonated at δ 3.14-3.34 as a multiplet for compounds (129-132). The (4-H) proton resonated at δ 3.37-3.52 as a double triplet for compound (129) or as a multiplet as in compounds (130-132). On the other hand, the (3-H) proton of the corresponding diastereoisomers of group B (133-135) resonated further downfield at δ 3.31-3.61 as a multiplet overlaping with (4-H) proton signals as a result of inversion of the configuration of (C-3).

Assignment of $^{13}$C NMR spectra of the final *novel* compounds depended on a previous study made on the proton-coupled $^{13}$C and $^1$H NMR spectroscopy of 2,4-diaminopyrimidines.[184] In case of group A (129-132) and group B (133-135), the three carbon atoms of the pyrimidine ring (C-2, C-4 and C-6) adjacent to ring nitrogens (some bearing amino group) should experience considerable deshielding and resonances of all three appeared in the range $\delta_C$ 160-166. The (C-5) carbon, which is not adjacent to nitrogen, gave a resonance at $\delta_C$ 105-109.

By comparing $^{13}$C NMR and DEPT spectra of compound (129) representing group A and compound (133) for group B, it was found that the two chiral carbons (C-3 and C-4) of the aliphatic side chain resonated at different values. In case of compound (129) one of them resonated at $\delta_C$ 71.35 while the second carbon resonated at $\delta_C$ 74.11. On the other hand, the corresponding carbon atoms of compound (133) resonated at $\delta_C$ 73.61 and $\delta_C$ 73.64 because of inversion of configuration of (C-3) carbon in group B compounds.

The specific rotations [α] for both group A and B compounds were calculated as group A compounds rotated the plane of polarised light to the left and designated (-) while their enantiomers in group B rotated the plane of polarised light to the right and designated (+). Surprisingly compound (129) has α = zero°, even by trying different concentrations and solvents. It may be a result of rotation of the plane of polarised light due to one of the chiral centre was coincidentally equal but in opposite direction to the rotation resulted from the second chiral centre. It can not be

a racemic mixture from the applied synthetic pathway as indicated by the pure NMR spectra that indicated the presence of one product along the synthetic pathway.

Finally, the expected chemical structures of the entire final *novel* compounds were confirmed by the correct mass and accurate mass spectra.

### 3.12 Conclusions

The active sites of Mtb-DHFR and h-DHFR have been studied to explore the glycerol A pocket as a new avenue for selectivity of new inhibitors. The glycerol A pocket was present only in Mtb-DHFR and was absolutely absent in the human enzyme. Accordingly four groups of target compounds (A, B, C and D) were designed with different side chains at 6-position of 2,4-diaminopyrimidine nucleus.

The synthetic pathway started by condensation of different derivatives of phenylacetonitrile with esters or lactones using bis(trimethylsilyl)lithium amide as a base to afford the corresponding β-ketonitriles. Diazomethane was used for O-methylation of β-ketonitrile derivatives to afford the expected enol ethers which in turn cyclised with guanidine to afford the corresponding 2,4-diaminopyrimidine derivatives. The final step was the deprotection of the hydroxy groups for group A, B and D. the deprotection of 1,2-diols were easily achieved by acid catalysis of aqueous TFA (30%) but the cleavage of benzyl ether linkage was robust due to the multifunctional groups present on the prepared products. Many deprotection procedures have been applied and the only compatible procedure was the use of anhydrous iron (III) chloride.

Consequently, an alternative approach was to condense the corresponding ester (47) without protecting the primary hydroxy group with phenyacetonitrile derivatives. Fortunately, ester (47) did condense well with phenylacetonitrile derivatives to form the corresponding β-ketonitriles which were O-methylated with diazomethane to form the corresponding enol ethers which, in turn, were cyclised with guanidine to afford the corresponding 2,4-diaminopyrimidine derivatives. By on-step deprotection of the ketal linkage, the latter afforded the target compounds of

group A. The products obtained from the two different starting esters (47) and (48) respectively have identical physical and spectroscopic properties.

# 4. EVALUATION OF TARGET COMPOUNDS

## 4.1 Introduction

Screening of drugs with *M. tuberculosis* directly is slow and requires biosafety Level 3 facilities and procedures.[185] The handling of specimens and cultures exposes laboratory staff to a serious risk of infection. Safety measures are essential, these include containment laboratories, approved safety cabinets and centrifuges, protective clothing, hand basins and facilities for the safe disposal of contaminated waste. All staff should be vaccinated with BCG vaccine, unless they are tuberculin positive, and must have medical supervision as determined by local policies.[12]

The slow growth rate of *M. tuberculosis* has been a puzzling and frustrating phenomenon for clinicians, technologists and public health authorities. In Koch's original report in 1882, he noted that microcolonies appear on the medium gradually during the third week of incubation. Over 100 years later, remarkably, most of the laboratories around the United States and the world still employ cultivation techniques that require three to six weeks to achieve growth. In substantial measure, this reflects the slow generation time inherent in the tubercle bacillus.[186]

An alternative strategy for evaluation of Mtb-DHFR inhibitors has been developed: initial screening in an engineered strain of the budding yeast *Saccharomyces cerevisiae* that is dependent on the *M. tuberculosis* DHFR for its growth. By using this system, we could screen all the target compounds as Mtb-DHFR inhibitors.[185] Furthermore, by using another engineered strain of the yeast that is dependent on the human DHFR for its growth, we could test the selectivity of the target compounds.

Genetic engineering is the deliberate modification of an organism's genetic information by directly changing its nucleic acid genome and is accomplished by a collection of methods known as recombinant DNA technology. First, the DNA responsible for a particular phenotype is identified and isolated. Once purified, the

gene or genes are fused with other pieces of DNA to form recombinant DNA molecules. These are propagated (gene cloning) by insertion into an organism that need not even to be in the same kingdom as the original gene donor. In gene cloning the new gene is inserted into a so-called vector that is inserted into the living cell. Vectors are usually plasmids, bacteriophages (viruses that specifically infect bacteria) and cosmids (a hybride between a bacteriophage and a plasmid that has been specially synthesised for use in gene cloning).[187]

Plasmids are small, circular molecules that can exist independently of host chromosome and are present in many bacteria and they are also present in some yeasts and other fungi. They have their own replication origins and are autonomously replicating and stably inherited. Plasmids have relatively few genes, generally less than 30. Their genetic information is not essential to the host, and bacteria that lack them usually function normally. Single-copy plasmids produce only one copy per host cell. Multicopy-plasmids may be present at concentrations of 40 or more per cell. Characteristically, plasmids can be eliminated from host cells in a process known as curing. Curing may occur spontaneously or be induced by treatments that inhibit plasmid replication while not affecting host cell reproduction. The inhibited plasmids are slowly diluted out of the growing bacterial population. Some commonly used curing treatments are UV and ionising radiation, thymine starvation and growth above optimal temperatures.[187] Plasmids may be cleaved at specific points by restriction enzymes, the new gene being inserted into the cut and joined to the original DNA by DNA ligase to form so-called recombinant DNA. The new plasmid is reinserted into the bacteria where bacterial reproduction resulted in the production of more recombinant DNA molecules.[188]

The slow growth and difficulty of working with mycobacteria led Gerum and her group to develop a system that could more quickly and safely screen potential inhibitors of the *M. tuberculosis* DHFR. As a first step, they constructed a yeast strain dependent on the *M. tuberculosis* DHFR for growth. The TH5 strain of yeast lacks endogenous expression of DHFR and is maintained by supplementing the medium with dTMP and a full complement of amino acids, uracil and adenine. To construct isogenic strain whose growth and viability depend on genetic complementation by plasmid-borne *M. tuberculosis* DHFR gene, *M. tuberculosis*

DHFR *dfrA* gene was cloned into different yeast vectors and then transformed into the TH5 strain of yeast and tested for their ability to grow without dTMP and for inhibition by DHFR-inhibitors.

The optimal strain for the screening of DHFR inhibitors must express the enzyme at a level high enough that the yeast can grow without supplementation but low enough that a proportional decrease in growth is achieved when an inhibitor of DHFR function is added. The strain that carried *dfrA* gene on p414CYC1 vector drove an intermediate level of DHFR and so this plasmid was chosen for use in all subsequent experiment and was designated TB-yeast.

*S. cerevisiae* has several advantages that have been exploited to develop similar systems for screening inhibitors of DHFR enzymes from different species such as *P. falciparum* and *Pneumocystis carinii.*[189] The simple yeast system offers the possibility to screen rapidly for inhibitors of the *M. tuberculosis* DHFR. The heterologous system circumvents the slow growth of *M. tuberculosis* and health risks inherent in working with mycobacteria.[185]

One might have chosen to screen these drugs in *Mycobacterium smegmatis,* taking advantage of its rapid growth (the growth rate is about equal to *S. cerevisiae*) and ease of genetic manipulation relative to those for *M. tuberculosis.* However, drug screening in *M. smegmatis* has not always been an accurate predictor of activity[190] or of the mechanism of action in *M. tuberculosis.*[191]

In addition, *S. cerevisiae* has several advantages. Firstly, plasmids that carry a centromere (a key component of a chromosome which is required for the chromosome to be distributed correctly to the daughter cells during cell division)[192] are maintained at one copy per cell in yeast, so the level of expression of an introduced gene can be controlled, even on a plasmid. In bacterial systems, control of plasmid number is far less precise and integration of genes into the chromosome would be required for equivalent studies to be carried out in *M. smegmatis.* Secondly, a series of yeast centromere plasmids that carry promoters that drive different levels of expression of linked genes had already been created.[193] These plasmids were used

to identify rapidly yeast clones that express the heterogeneous enzyme at a level convenient for drug screening. Lastly, homologous recombination is highly active in *S. cerevisiae* and foreign genes can be integrated into a plasmid simply by cotransformation with a gapped plasmid, a significant advantage when a large number of different alleles are to be tested.[185]

To use the complementation system to screen antifolate drugs, it was important that the heterologus DHFRs be expressed at an extremely low level so that inhibition of the enzyme would be reflected as a proportional decrease in yeast growth. Preliminary work had shown that the Pf-yeast strain expressed the plasmodium DHFR domain at very low levels and that the growth of that strain was strongly inhibited by the potent inhibitor MTX. Unexpectedly, however, even high levels of MTX did not inhibit the growth of any other complemented strains. For example human-yeast (Hu-yeast) grew normally in MTX concentrations as high as (1 μM) even though the $IC_{50}$ of the purified human DHFR is about (1 nM). As MTX binds essentially stoichiometrically to human DHFR, the amount of MTX needed to inhibit the growth of yeast dependent on heterologous DHFR expression is proportional to the cellular DHFR concentration. The insensitivity of many of the complemented yeast strains to MTX resulted from high DHFR levels and it would be necessary to reduce DHFR expression to enhance sensitivity of the system which resulted in a MTX $IC_{50}$ of approximately (3 μM ) for the human yeast.[189]

The $IC_{50}$ that one measures for the yeast system is a complex function of the level of Mtb-DHFR enzyme, the level of penetration of the yeast by the drug tested and the actual level of inhibition of the enzyme within the cell. For that reason, the absolute $IC_{50}$ cannot predict the MIC for *M. tuberculosis*. However, the relative inhibition of yeast growth by related series of the inhibitors is valuable information for drug testing. Further work to develop these compounds will require working with mycobacterial cultures, purified enzyme and animal models of tuberculosis infection but we have reduced the amount of initial work necessary to identify drug candidates by working in this yeast system.[185]

To increase the sensitivity of this yeast system, we included sulfanilamide (1.0 mM) in the growth medium for all yeast assays. Sulfa drugs inhibit

dihydropteroate synthase, an enzyme upstream of DHFR in the folate pathway.[194] The sulfa drugs are always used in combination with DHFR inhibitors because the two drugs work in synergy in many pathogens. In this case, they increase the effectiveness of the DHRF inhibitors in the heterologous yeast system and facilitate the comparative screening. The addition of sulfanilamide to these assays does not compromise the results, since we are measuring the relative inhibition of a set of closely related compounds. In any case, it is likely that a sulfa drug would be part of a combination therapy which included a DHFR inhibitor, for the treatment of tuberculosis.[185,189]

By using the Hu-yeast and the TB-yeast strains, we could perform a qualitative spoke assay for determination of the efficacies of the prepared target compounds as DHFR inhibitors. The spoke assays were useful for rapid classification of test compounds into categories of approximate potency and selectivity.[185,195]

The spoke assay-based screening of the activities of the test compounds against TB-yeast, Hu-yeast and the wild-type *S. cerevisiae* was performed as following:

1- Sulfanilamide was spread onto fresh plates and allowed to absorb into the medium overnight. The medium contains 10% yeast extract, 10% peptone and 10% dextrose.

2- By using the narrow side of the flat end of a sterile toothpick, pick up some yeast cells from a plate. Starting from the center of a fresh plate, drag the toothpick in a straight line outward toward the edge of the plate. Continue with other strains on the same plate, creating a wheel spoke pattern. Incubate the plate until the yeast has grown well.

3- Replica plate onto a fresh plate.

4- Replica immediately from the second plate onto additional fresh plate.

5- Place the drug in solution (10 µl of a 10 mM solution in DMSO) in the center of the plate and let the plate absorb it (about 5 min).

The last plate should contain no drug and serve as a control for transfer of cells. Incubate at 30 °C for 3 days.

6- Measure the distance from the center of the plate to the beginning of growth for each spoke (the inhibition zone). The inhibition zones were calculated from the mean of three different experiments. The greater the distance the more sensitive the strain

is to the drug. The inhibition zones that were at least 4.0 mm more than that from the control plate with DMSO alone, were scored as inhibited by the compound on the plate.[185,195]

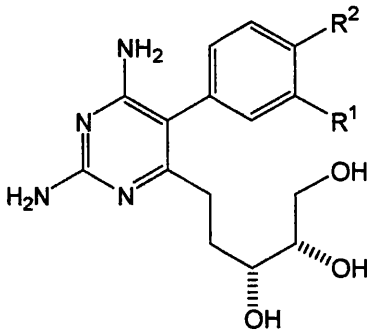
## 4.2 Results and Discussion

Trimethoprim was used for comparison purposes as it is reported to be a highly selective inhibitor of bacterial DHFR. Model compounds were tested as well because pyrimethamine had shown some activity *in vitro* against the *M. avium* DHFR complex.[185,195]

The synthesised *novel* target compounds were evaluated for inhibitory activity and selectivity against Mtb-DHFR *in vitro* by the radial spoke assay with three different yeast strains, the TB-yeast, the Hu-yeast and the wild-type *S. cerevisiae*. The measured inhibition zones would be compared with that obtained from trimethoprim and model compounds. All compound assays with the TB-yeast contained sulfanilamide (1.0 mM) in the growth medium to improve sensitivity to the DHFR inhibitors. DMSO was used as a negative control to ensure that the solvent and sulfanilamide (1.0 mM) were not inhibiting the yeast growth non-specifically.[185,189] The same method was applied to the Hu-yeast strain and the wild-type *S. cerevisiae* to test the selectivity of the target compounds.

The results of the radial spoke assay performed for the Hu-yeast, the TB-yeast strains as well as the wild-type *S. cerevisiae* are represented in tables (3-6). The relative potency and selectivity of the tested compounds against Mtb-DHFR were determined by measuring the diameters of the inhibition zones for the tested yeast strains and comparing these values with that obtained from a control plate containing DMSO only.

For group A compounds (table 3), compound **129** was the most active compound against the TB-strain and was inactive against both the Hu-yeast strain and the wild-type *S. cerevisiae*. Compound **130** had lower activity against the TB-yeast and was also inactive against the Hu-yeast and the wild-type *S. cerevisiae*. Compounds **131** and **132** were inactive against all types of the tested strains.

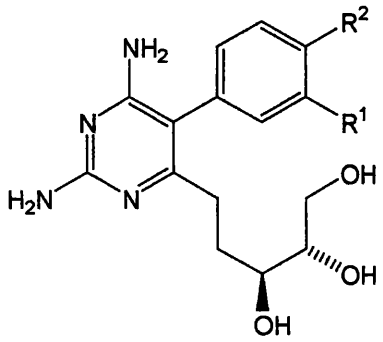



| Compound no. | $R^1$ | $R^2$ | Inhibition zone diameter (nearest mm) and (SD) TB-yeast | Inhibition zone diameter (nearest mm) and (SD) Hu-yeast | Inhibition zone diameter (nearest mm) and (SD) Wild-type *S. cerevisiae* |
|---|---|---|---|---|---|
| 129 | H | H | 11 (0.57) | 6 (1.0) | 6 (0.0) |
| 130 | H | Cl | 9 (0.57) | 7 (0.57) | 7 (0.57) |
| 131 | H | Br | 7 (1.0) | 6 (0.57) | 8 (1.0) |
| 132 | Cl | Cl | 8 (0.57) | 8 (1.0) | 8 (0.57) |
| DMSO | | | 5 (0.57) | 6 (0.57) | 5 (1.0) |

**Table (3) Structures and inhibition zone diameter of group A target compounds against the Hu-yeast, the TB-yeast and the wild-type *S. cerevisiae*. SD, standard deviation**

Interestingly, compound **129** was the best in term of potency and selectivity against TB-yeast among the member of this group of inhibitors.

In case of group B of target compounds (table 4), only compound **133** was equipotent to compound **130** from group A against the TB-yeast and was inactive against the Hu-yeast and the wild type *S. cerevisiae* as well. Compounds **134** and **135** were inactive against the three types of DHFR enzymes.



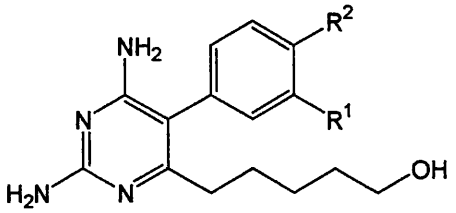

| Compound no. | $R^1$ | $R^2$ | Inhibition zone diameter (nearest mm) and (SD) TB-yeast | Inhibition zone diameter (nearest mm) and (SD) Hu-yeast | Inhibition zone diameter (nearest mm) and (SD) The wild-type *S. cerevisiae* |
|---|---|---|---|---|---|
| 133 | H | H | 9 (0.57) | 8 (1.0) | 8 (0.0) |
| 134 | H | Cl | 7 (0.57) | 7 (0.57) | 6 (0.57) |
| 135 | H | Br | 8 (0.0) | 5 (0.57) | 6 (0.0) |
| DMSO | | | 5 (0.57) | 6 (0.57) | 5 (1.0) |

**Table (4) Structures and inhibition zone diameter of group B target compounds against the Hu-yeast, the TB-yeast and the wild-type *S. cerevisiae*. SD, standard deviation**

As expected, compound **129** that has the configuration of the chiral carbons on the side chain at 6-position of the pyrimidine ring that resemble that of the glycerol molecule in the active site of Mtb-DHFR was essential requirement for potency. By changing the configuration of one chiral carbon (C-3) as in compound (133) resulted in decreasing the potency but retain the selectivity as it is still inactive against the Hu-yeast.

For group C of target compounds (table 5), compounds **107** and **109** were weak inhibitors to wild-type *S. cerevisiae* and were inactive against the TB-yeast and the Hu-yeast strains. Compound **110** showed an equipotent inhibition to compound

**130** from group A against the TB-yeast while compound **108** was inactive against all the tested strains.

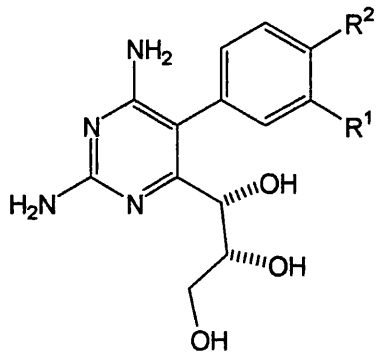



| Compound no. | $R^1$ | $R^2$ | Inhibition zone diameter (nearest mm) and (SD) TB-yeast | Inhibition zone diameter (nearest mm) and (SD) Hu-yeast | Inhibition zone diameter (nearest mm) and (SD) The wild-type *S. cerevisiae* |
|---|---|---|---|---|---|
| 107 | H | H | 7 (0.0) | 7 (0.0) | 9 (0.57) |
| 108 | H | Cl | 8 (0.0) | 8 (0.57) | 8 (0.57) |
| 109 | H | Br | 8 (0.57) | 8 (0.57) | 9 (0.57) |
| 110 | Cl | Cl | 9 (0.57) | 9 (0.0) | 9 (057) |
| DMSO | | | 5 (0.57) | 6 (0.57) | 5 (1.0) |

**Table (5) Structures and inhibition zone diameter of group C target compounds against the Hu-yeast, the TB-yeast and the wild-type *S. cerevisiae*. SD, standard deviation**

The absence of the chiral carbons and the hydroxy groups had a dramatic negative effect on potency and selectivity of group C compounds, which reflect their great contribution on improving potency and selectivity of these classes of inhibitors.

All members of group D target compounds (table 6) were inactive against all the tested yeast strains.

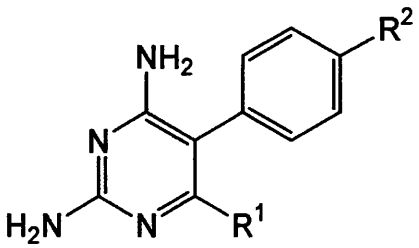



| Compound no. | $R^1$ | $R^2$ | Inhibition zone diameter (nearest mm) and (SD) TB-yeast | Inhibition zone diameter (nearest mm) and (SD) Human-yeast | Inhibition zone diameter (nearest mm) and (SD) The wild-type *S. cerevisiae* |
|---|---|---|---|---|---|
| 125 | H | H | 5 (0.57) | 5 (0.0) | 5 (0.0) |
| 126 | H | Cl | 5 (0.0) | 5 (0.57) | 5 (0.57) |
| 127 | H | Br | 5 (0.0) | 5 (0.57) | 5 (0.57) |
| 128 | Cl | Cl | 8 (0.57) | 8 (0.0) | 7 (0.57) |
| DMSO | | | 5 (0.57) | 6 (0.57) | 5 (1.0) |

**Table (6) Structures and inhibition zone diameter of group D target compounds against the Hu-yeast, the TB-yeast and the wild-type *S. cerevisiae*. SD, standard deviation**

This result reflected that the length of the carbon skeleton of the side chain at the 6-position of the pyrimidine ring was also an important parameter for perfect binding to the glycerol pocket in the active site of Mtb-DHFR. Compounds of group D were inactive compared to compounds of group A as a result of shortening the side-chain at the 6-position although they were still having the chiral carbons and the hydroxy groups.

In case of trimethoprim and model compounds (table 7), all were inactive against TB-yeast, human-yeast and *S. cerevisiae* as well.




| Compound no. | $R^1$ | $R^2$ | Inhibition zone diameter (nearest mm) and (SD) TB-yeast | Inhibition zone diameter (nearest mm) and (SD) Human-yeast | Inhibition zone diameter (nearest mm) and (SD) The wild-type *S. cerevisiae* |
|---|---|---|---|---|---|
| 101 | $CH_3$ | H | 7 (0.57) | 8 (0.0) | 8 (0.57) |
| 102 | $CH_2CH_2Ph$ | H | 5 (0.0) | 5 (0.57) | 5 (0.57) |
| 103 | $CH_2CH_3$ | Cl | 5 (1.0) | 6 (1.0) | 5 (0.57) |
| Trimethoprim | | | 5 (0.57) | 6 (0.0) | 6 (0.0) |
| DMSO | | | 5 (0.57) | 6 (0.57) | 5 (1.0) |

**Table (7) Structures and inhibition zone diameter of model compounds and trimethoprim against the Hu-yeast, the TB-yeast and the wild-type *S. cerevisiae*. SD, standard deviation**

The results from the radial assays revealed that the new designed functional group at the 6-position of the pyrimidine ring not only successfully increased the potency against the TB-yeast compared with trimethoprim and model compounds but also retained their selectivity by being inactive against the human and the wild-type *S. cerevisiae* DHFR.

Inversion of configuration of one chiral carbon in case of group B target compounds alters the potency but does not affect the selectivity. On the other hand, disappearance of the two secondary hydroxy groups as in case of group C target compounds seriously affects both the potency and selectivity as some members of this group of compounds showed activity against the wild-type *S. cerevisiae* DHFR and most of them lost their activity against the TB-yeast.

Shortening of the carbon skeleton of the side chain at the 6-position of the pyrimidine ring had a dramatic effect on potency as all members of group D target compounds were inactive against all types of tested enzymes.

Regarding the effect of the side-chain at 5-position of the pyrimidine ring, the presence of the 4-chloro substituent at the phenyl ring reduced the activity of group A and B compounds as TB-DHFR inhibitors. On the other hand the 4-bromo and the 3,4-dichloro analogues lost their activity as DHFR inhibitors.

## 4.3 Conclusion

Screening of drugs with *M. tuberculosis* cultures is slow and requires biosafety Level 3 facilities and procedures. We have used an alternative strategy by initial screening of the prepared target compounds in two types of engineered strains of the budding yeast that are dependent on the *M. tuberculosis* DHFR and human DHFR respectively for their growth.

By using this system, we have screened all the prepared target compounds and found that three compounds **129**, **130** and **133** showed higher potency and selectivity as Mtb-DHFR inhibitors compared to trimethoprim and model compounds. Disappearance of the two chiral centers or shortening of the length of the carbon skeleton of the side chain at 6-position made these compounds inactive. Inversion of the configuration of one chiral carbon has reduced the potency but does not affect the selectivity.

Introduction of a halogen to the phenyl side chain at 5-position of the pyrimidine ring reduces the potency and selectivity although it ncreases the lipophilicity of the target compounds and consequently should enhance the potency. It might have another influence once tested on the *M. tuberculosis* itself as it might enhance the penetration of the highly lipophilic cell wall of the mycobacteria. Thus, further study of their efficacies against the mycobacteria is likely to be a fruitful avenue of research.

These results reveal that we have successfully designed and synthesised a new functional group that provid selectivity and potency to the 2,4-diaminopyrimidine pharmacophore as inhibitors to Mtb-DHFR. Consequently, compounds of group A are good potential leads for a further development of potent and selective inhibitors against *M. tuberculosis* DHFR.

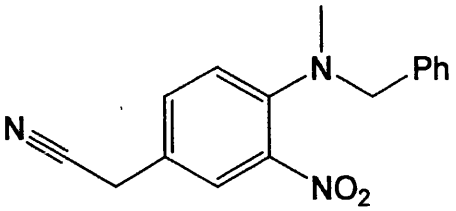
# 5. EXPERIMENTAL

## 5.1 Chemistry

### General Synthetic Procedures

$^{1}H$ and $^{13}C$ NMR spectra were recorded on JEOL/Varian GX270 and EX400 spectrometers. NMR spectra are reported in ppm downfield from tetramethylsilane. Samples were dissolved in $CDCl_3$, unless otherwise stated. Coupling constants are reported in (Hz). FAB mass spectra in [3-nitrobenzyl alcohol (3-NBA)] were obtained using a VG7070E spectrometer. IR spectra ($cm^{-1}$) were measured on neat compounds (for oil products) or as KBr discs (for solid products) on the Perkin-Elmer RXI FT-IR spectrometer. Optical rotations were measured in a 1 dm cell on an Optical Activity Ltd polarimeter and concentration (c) is expressed in g/100 ml. Chromatography was performed on silica gel (BDH, 40-63 μm). The synthesised compounds were purified by chromatography under gravity or a light pressure applied to the column. Reactions were monitored by TLC using pre-coated aluminum sheet (Merck, silica 60 $F_{254}$) and were visualized with UV light at 254 nm, solutions of 10% sulfuric acid, ninhydrin or iron (III) chloride. TLC and $R_f$ values were performed on the same mobile phase as the column unless otherwise stated. All reactions were carried out under a static atmosphere of nitrogen and stirred magnetically at room temperature unless otherwise stated. Solvents were evaporated under reduced pressure using a Büchi rotary evaporator. Chemicals were supplied by Aldrich Chemical Company (Poole, Dorset. UK) or Fluka. Reagent-grade solvents were utilised as received except for THF which was freshly distilled under nitrogen from Na/benzophenone. Melting points were determined by using a Reichert-Jung Thermo Galen instrument and are uncorrected.

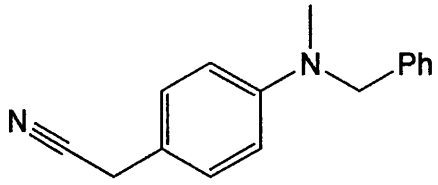
**4-(N-Benzyl-N-methylamino)-3-nitrophenylacetonitrile (19)**




**(19)**

N-Benzylmethylamine (1.2 g, 10 mmol) was added to a solution of compound (31) (0.40 g, 2 mmol) in dry THF (5 ml) under nitrogen. The reaction mixture was boiled under reflux overnight. The cooled brown solution was diluted with diethyl ether and filtered. The resulting solution was concentrated and purified by chromatography (diethyl ether/pet. ether (40-60 °C)/dichloromethane, 3:4:3) to afford (19) (0.46 g, 81%) as a pale yellow oil; IR $\nu_{max}$ 2361 (CN), 1438, 1375 ($NO_2$); NMR $\delta_H$ 2.08 (3H, s, $CH_3$), 3.69 (2H, s, $CH_2CN$), 4.42 (2H, s, $CH_2N$), 7.05 (1H, d, $J$ = 9.0 Hz, 5-H), 7.32-7.36 (6H, m, 6-H + Ph-$H_5$), 7.72 (1H, d, $J$ = 2.0 Hz, 2-H); MS *m/z* 282.1230 (M + H) ($C_{16}H_{16}N_3O_2$ requires 282.1242), 204 (M – $C_6H_5$), 91 (Bn).

**4-(N-Benzyl-N-methylamino)phenylacetonitrile (20)**

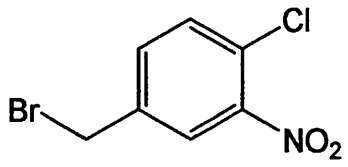



**(20)**

Triethylamine (1.3 g, 13 mmol) was added to a solution of compound (36) (6.5 g, 18 mmol) in dry acetonitrile (60 ml) under nitrogen, followed immediately by the addition of tetraethylammonium cyanide (3.3 g, 21 mmol). After stirring overnight the reaction mixture was evaporated and the remaining residue was extracted with a mixture of diethyl ether and pet. ether (40-60 °C) (50%). The ether solution was evaporated and the remaining residue was purified by chromatography (diethyl ether/

pet. ether (40-60 °C)/chloroform, 1:6:1) to afford (20) (2.0 g, 40%); $R_f = 0.58$ as a pale yellow oil; NMR $\delta_H$ 2.95 (3H, s, $CH_3$), 3.94 (2H, s, $CH_2CN$), 4.46 (2H, s, $CH_2N$), 6.65 (2H, d, $J = 8.6$ Hz, 3,5-$H_2$), 7.01 (2H, d, $J = 8.6$ Hz, 2,6-$H_2$), 7.04-7.27 (5H, m, Ph-$H_5$); MS *m/z* 237.1385 (M + H) ($C_{16}H_{17}N_2$ requires 237.1391), 210 (M – CN), 194 (M – $C_2H_3N$), 91 (Bn).

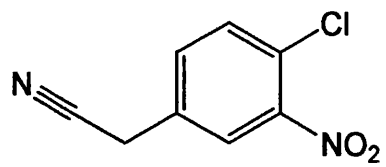
**4-Chloro-3-nitrobenzyl bromide (30)**




**(30)**

4-Chloro-3-nitrotoluene (31.0 g, 0.18 mmol) was added to a suspension of N-bromosuccinimide (31.0 g, 0.18 mmol) in carbon tetrachloride (200 ml) and dibenzoyl peroxide (0.65 g, 2.7 mmol) was added. The reaction mixture was heated at reflux overnight. The reaction mixture was cooled, filtered and the solvent was concentrated. The residue was purified by chromatography (diethyl ether/pet. ether (40-60 °C)/dichloromethane, 3:4:3) to give (30) (29.0 g, 65%) as a reddish brown oil; NMR $\delta_H$ 4.48 (2H, s, $CH_2Br$), 7.52 (1H, d, $J = 8.3$ Hz, 5-H), 7.56 (1H, dd, $J = 8.3$, 1.7 Hz, 6-H), 7.91 (1H, d, $J = 1.7$ Hz, 2-H); MS *m/z* 250 (M - H) $C_8H_6{}^{37}Cl^{79}BrN_2O_2$, 248 (M - H) $C_8H_6{}^{35}Cl^{79}BrN_2O_2$.

**4-Chloro-3-nitrophenylacetonitrile (31)**

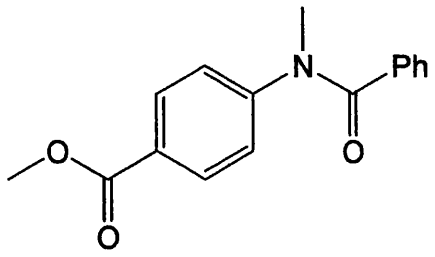



**(31)**

Tetraethylammonium cyanide (0.50 g, 3.2 mmol) was added to compound (30) (0.66 g, 2.6 mmol) in dry acetonitrile (10 ml) under argon. The resulting deep green

solution was stirred for 4 h. the solvent was removed under vacuum and the residue was purified by chromatography (hexane/diethyl ether, 1:2) to afford (31) (0.15 g, 29%) as a pale yellow oil; IR $\nu_{max}$ 2253 (CN), 1536, 1356 ($NO_2$), 806 (C-Cl) $cm^{-1}$; NMR $\delta_H$ 3.85 (2H, s, $CH_2CN$), 7.54 (1H, dd, $J$ = 8.2, 2.0 Hz, 6-H), 7.60 (1H, d, $J$ = 8.2 Hz, 5-H), 7.86 (1H, d, $J$ = 2.0 Hz, 2-H); MS *m/z* 197 (M - H) $C_8H_6{}^{37}ClN_2O_2$, 195 (M - H) $C_8H_6{}^{35}ClN_2O_2$, 350/348 (M + 3-NBA).

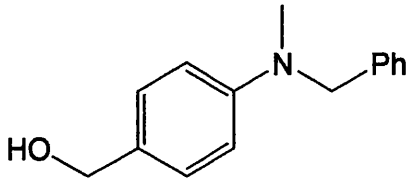
**Methyl 4-(N-benzoyl-N-methylamino)benzoate (34)[130]**




**(34)**

Benzoyl chloride (7.7 g, 55 mmol) was added to a solution of methyl 4-(N-methylamino)benzoate (9.0 g, 55 mmol) and triethylamine (8 ml) in dry diethyl ether (200 ml). After stirring for 1 h the ether solution was washed with water, dried ($MgSO_4$) and then evaporated. The remaining residue was purified by recrystallisation from aqueous ethanol to afford (34) (12.13 g, 90%) as a buff solid: mp 58-60 °C (lit.[130] mp 57-60 °C); NMR $\delta_H$ 3.51 (3H, s, $CH_3N$), 3.85 (3H, s, $CH_3O$), 7.06 (2H, d, $J$ = 8.8 Hz, 3,5-$H_2$), 7.13-7.28 (5H, m, Ph 3,4,5-$H_3$ + 2,6-$H_2$), 7.86 (2H, d, $J$ = 8.8 Hz, Ph 2,6-$H_2$).

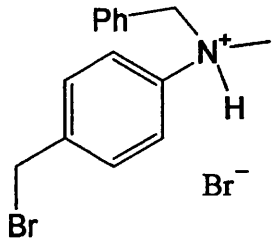
**4-(N-Benzyl-N-methylamino)benzyl alcohol (35)[131]**




**(35)**

Compound (34) (23.0 g, 85 mmol) dissolved in dry diethyl ether (100 ml) was added slowly to a stirred suspension of $LiAlH_4$ (6.5 g, 170 mmol) in dry diethyl ether (200 ml). The reaction mixture was stirred at 35 °C for 4 h. The reaction mixture was cooled and quenched by the addition of ice cold water (100 ml) followed by extraction with ethyl acetate (2 × 200 ml). The organic layer was separated, washed with water, dried ($MgSO_4$) and then evaporated to dryness. The remaining residue was purified by chromatography (diethyl ether/pet. ether (40-60 °C), 1:1) to give (35) (10.4 g, 54%): $R_f$ = 0.39 as a pale yellow oil; NMR $\delta_H$ 2.21 (1H, t, $J$ = 5.1 Hz, OH), 2.95 (3H, s, $CH_3$), 4.44 (2H, d, $J$ = 5.1 Hz, $CH_2OH$), 4.46 (2H, s, $CH_2N$), 6.65 (2H, d, $J$ = 8.8 Hz, 2,6-$H_2$), 7.12 (2H, d, $J$ = 8.8 Hz, 3,5-$H_2$),7.14-7.26 (5H, m, Ph-$H_5$).

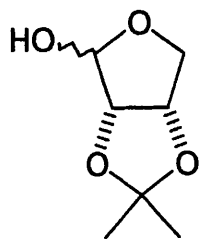
**N-Benzyl-4-bromomethyl-N-methylanilinium bromide (36)[131]**




**(36)**

An excess of hydrobromic acid in acetic acid (30%, 20 ml) was added to compound (35) (4.5 g, 20 mmol) under nitrogen. After 6 h the reaction mixture was evaporated to afford (36) (6.2 g, 84%) as a hygroscopic orange solid; NMR $\delta_H$ 3.23 (3H, s, $CH_3$), 4.40 (2H, s, $CH_2Br$), 4.71 (2H, s, $CH_2N$), 7.24-7.35 (5H, m, Ph-$H_5$), 7.39 (2H, d, $J$ = 8.0 Hz, 2,6-$H_2$), 7.54 (2H, d, $J$ = 8.0 Hz, 3,5-$H_2$).

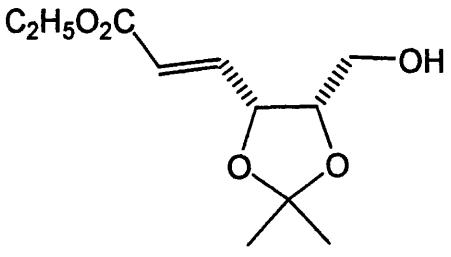
**2,3-O-Isopropylidene-L-erythrose (44)[136]**



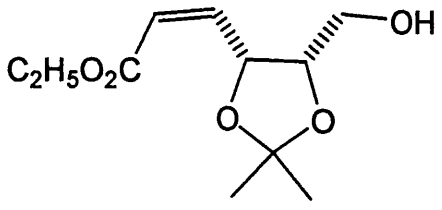

**(44)**

To L-arabinose (10.0 g, 67 mmol) and 2,2-dimethoxypropane (23.0 g, 0.22 mol) in dry DMF (130 ml) was added 4-toluenesulfonic acid (150 mg) and the reaction mixture was stirred for 90 min. The mixture was neutralised with solid sodium carbonate and evaporated to dryness. The residue was partitioned between water (120 ml) and pet. ether (40-60 °C) (60 ml). Sodium periodate (35.5 g, 0.17 mol) was added to the aqueous layer and the reaction mixture was stirred for 2 h. Solid sodium carbonate was added and the slurry was stirred for 1 h. Extraction with ethyl acetate and evaporation gave a residue, which was extracted with dichloromethane. The pale yellow oil that remained after evaporation of dichloromethane was purified by chromatography (diethyl ether/pet. ether (40-60 °C), 2:1) to give (44) (5.8 g, 54%); $R_f$ = 0.68; $[\alpha]^{20}_D$ = +20° (c 5.3, $CHCl_3$) (lit.[133] $[\alpha]^{25}_D$ = +75° (c 1.0, $CH_3OH$)) as a colourless oil; NMR $\delta_H$ 1.31 (3H, s, Me), 1.46 (3H, s, Me), 3.89 (1H, d, $J$ = 2.5 Hz, OH), 4.01 (1H, d, $J$ = 10.5 Hz, 4-H), 4.05 (1H, dd, $J$ =10.5, 3.5 Hz, 4-H), 4.55 (1H, d, $J$ = 6.0 Hz, 2-H), 4.82 (1H, dd, $J$ = 6.0, 3.5 Hz, 3-H), 5.39 (1H, d, $J$ = 2.5 Hz, 1-H); MS *m/z* 159.0650 (M + H) ($C_7H_{11}O_4$ requires 159.0657), 181 (M + Na), 101 (M – $C_3H_5O$).

**(*E*,*4S*,*5R*)-5-(Ethoxycarbonylethenyl)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (45) and (*Z*,*4S*,*5R*)-5-(ethoxycarbonylethenyl)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (46)[138]**




**(45)**

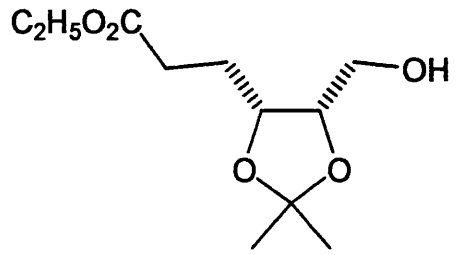



**(46)**

To ethyl triphenylphosphoranylidineacetate (9.3 g, 27 mmol) in dichloromethane (100 ml) was added compound (44) (2.9 g, 18 mmol) in dichloromethane (30 ml). The mixture was stirred overnight. Excess diethyl ether was added to the residue

remaining after evaporation of the dichloromethane. The formed white solid was filtered off and the ether was dried ($MgSO_4$), and concentrated. Purification by chromatography (diethyl ether/pet. ether (40-60 °C), 1:1) afforded first the *Z*-alkene (46) (2.2 g, 54%); $R_f$ = 0.50; $[\alpha]^{20}{}_D$ = -120° (c 3.7, $CHCl_3$), (lit.[142] $[\alpha]^{22}{}_D$ = -111.3° (c 1.5, EtOH)) as a colourless oil and then the *E*-isomer (45) (0.6 g, 15%); $R_f$ = 0.21; $[\alpha]^{20}{}_D$ = -32.8° (c 3.2, $CHCl_3$), (lit.[142] $[\alpha]^{22}{}_D$ = -6.6° (c 1.7, EtOH)) as a colourless oil. The *Z*-isomer was characterised with NMR $\delta_H$ 1.29 (3H, t, *J* = 7.0 Hz, $CH_2CH_3$), 1.40 (3H, s, Me), 1.53 (3H, s, Me), 2.44 (1H, dd, *J* = 7.4, 5.5 Hz, OH), 3.45 (1H, m, CH*H*OH), 3.59 (1H, m, C*H*HOH), 4.16 (2H, q, *J* = 7.4 Hz, $CH_2CH_3$), 4.53-4.57 (1H, m, 4-H), 5.58 (1H, dt, *J* = 7.1, 1.7 Hz, 5-H), 5.91 (1H, dd, *J* = 11.7, 1.7 Hz, $CHCO_2$), 6.36 (1H, dd, *J* = 11.7, 7.1 Hz, C*H*=CH). The *E*-isomer had NMR $\delta_H$ 1.29 (3H, t, *J* = 7.2 Hz, $CH_2CH_3$), 1.40 (3H, s, Me), 1.52 (3H, s, Me), 2.41 (1H, t, *J* = 5.9 Hz, OH), 3.55 (2H, t, *J* = 5.9 Hz, $CH_2OH$), 4.18 (2H, q, *J* = 7.2 Hz, $CH_2CH_3$), 4.33-4.38 (1H, m, 4-H), 4.79 (1H, dt, *J* = 5.5, 1.6 Hz, 5-H), 6.12 (1H, dd, *J* = 15.6, 1.6 Hz, $CHCO_2$), 6.88 (1H, dd, *J* = 15.6, 5.5 Hz, C*H*=CH); MS *m/z* 231.1240 (M + H) ($C_{11}H_{19}O_5$ requires 231.1232), 215 (M - $CH_3$), 173 (M – $C_3H_5O$), 143 (M – $C_4H_7O_2$).

**(4*S*,5*R*)-5-(Ethoxycarbonylethyl)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (47)**

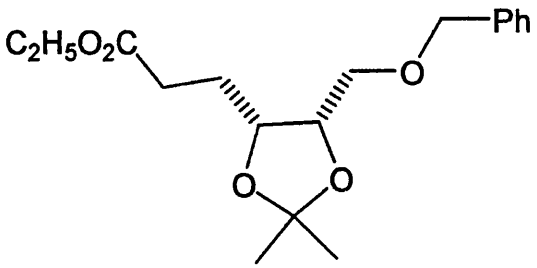



**(47)**

A mixture of the unsaturated esters (45) and (46) (2.3 g, 10 mmol) was stirred in ethanol (100 ml) with Pd/C (5%, 150 mg) under an atmosphere of hydrogen for 3h. The catalyst was removed by filtration through Celite®. The solvent was evaporated to afford (47) (2.3 g, 99%); $[\alpha]^{20}{}_D$ = +13.4° (c 2.5, EtOH), (lit.[142] $[\alpha]^{22}{}_D$ = +22.5° (c 2, EtOH)) as a pale yellow oil; NMR $\delta_H$ 1.26 (3H, t, *J* = 7.0 Hz, $CH_2CH_3$), 1.33 (3H, s, Me), 1.42 (3H, s, Me), 1.82 (2H, m, $CH_2CH$), 2.40 (1H, br, OH), 2.40 (1H, dt, *J* =

16.4, 7.8 Hz, $CHCO_2$), 2.53 (1H, dt, $J$ = 16.4, 7.4 Hz, $CHCO_2$), 3.65 (2H, d, $J$ = 5.1 Hz, $CH_2OH$), 4.09-4.20 (4H, m, 4-H + 5-H + $CH_2CH_3$); MS *m/z* 233.1396 (M + H) ($C_{11}H_{19}O_5$ requires 233.1388), 217 (M - $CH_3$), 201 (M - $CH_3O$), 175 (M – $C_3H_5O$).

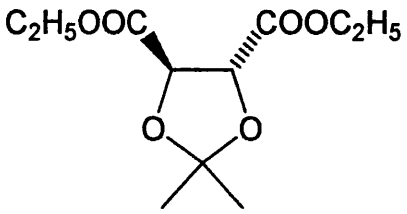
**(4*S*,5*R*)-4-Benzyloxymethyl-5-(ethoxycarbonylethyl)-2,2-dimethyl-1,3-dioxolane (48)**




**(48)**

Lithium bis(trimethylsilyl)amide (1 M in THF) (10 ml, 10 mmol) was added to (47) (2.3 g, 10 mmol) and benzyl bromide (3.4 g, 20 mmol) in dry DMF (5 ml). After 2h, the reaction was quenched by addition of water, followed by extraction with diethyl ether. The ether layer was washed with water and brine, dried ($MgSO_4$) and evaporated. Purification by chromatography (diethyl ether/pet. ether (40-60 °C), 1:4) afforded (48) (1.6 g, 48%); $R_f$ = 0.32; $[\alpha]^{20}_D$ = +24.8° (c 4.4, $CHCl_3$) as a pale yellow oil; NMR $\delta_H$ 1.24 (3H, t, $J$ = 7.0 Hz, $CH_2CH_3$), 1.33 (3H, s, Me), 1.42 (3H, s, Me), 1.72-1.86 (2H, m, $CH_2CH$), 2.34-2.40 (1H, m, $CHCO_2$), 2.48-2.54 (1H, m, $CHCO_2$), 3.47-3.54 (2H, m, $CH_2OBn$), 4.08-4.15 (3H, m, 5-H + $CH_2CH_3$), 4.28 (1H, dd, $J$ = 11.9, 6.1 Hz, 4-H), 4.50 (1H, d, $J$ = 12.1 Hz, CHPh), 4.57 (1H, d, $J$ = 12.1 Hz, CHPh), 7.24-7.33 (5H, m, Ph-$H_5$); MS *m/z* 323.1864 (M + H) ($C_{18}H_{27}O_5$ requires 323.1858), 265 (M – $C_3H_5O$), 91 (Bn).

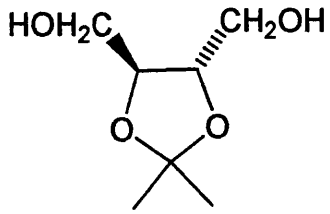
**Diethyl (*R*,*R*)-2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate (50)[140]**



**(50)**

Diethyl (*R*,*R*)-2,3-dihydroxybutanedioate (15 g, 70 mmol), 2,2-dimethoxypropane (8.0 g, 80 mmol), and 4-toluenesulfonic acid (66 mg, 0.34 mmol) in dichloromethane (200 ml) were boiled under reflux through activated 4 Å molecular sieves (33 g) in a Soxhlet apparatus for 3 h. Anhydrous sodium carbonate (83 mg, 1 mmol) was then added. Filtration, drying ($MgSO_4$) and evaporation gave (50) (16.0 g, 89%) as a pale brown oil. $[\alpha]^{20}_D$ = -29° (c 4.2, $CHCl_3$) (lit.[196] $[\alpha]^{20}_D$ = -49.4° (neat liquid)); NMR $\delta_H$ 1.32 (6H, t, *J* = 7.2 Hz, 2 × $CH_2CH_3$), 1.50 (6H, s, $CMe_2$), 4.28 (4H, q, *J* = 7.2 Hz, 2 × $CH_2CH_3$), 4.77 (2H, s, 4,5-$H_2$).

**(*S*,*S*)-4,5-Di(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane (51)[140]**

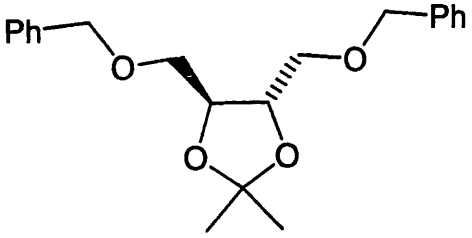


**(51)**

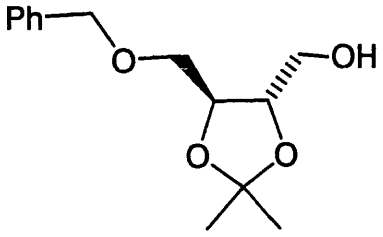
A suspension of $LiAlH_4$ (6.0 g, 150 mmol) in dry THF (60 ml) was heated for 30 min. A solution of (50) (18 g, 70 mmol) in dry THF (80 ml) was added over 1.5 h. The mixture was boiled under reflux for a further 5 h. It was then cooled to 0 °C and water (10 ml), NaOH solution (4 M, 10 ml), and water (30 ml) were added cautiously in turn. The aluminium salts were removed by filtration. Water and THF were removed under reduced pressure to give crude (51). The aluminium salts were then extracted with boiling dioxane (3 × 100 ml); removal of dioxane under reduced pressure gave further product (7.0 g, 60%) of (51) as a pale yellow oil. $[\alpha]^{18}_D$ = +7.5°

(c 4.4, ethanol) (lit.[197] $[\alpha]^{25}_D$ = +3.8° (c 3.7, $CHCl_3$)); NMR $\delta_H$ 1.41 (6H, s, $CMe_2$), 2.65 (2H, br, 2 × OH), 3.68-3.78 (4H, m, 2 × $CH_2$), 3.96-3.98 (2H, m, 4,5-$H_2$).

**(*S*,*S*)-4,5-Di(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolane (52) and (*S*,*S*)-4-benzyloxymethyl-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (53)[142]**



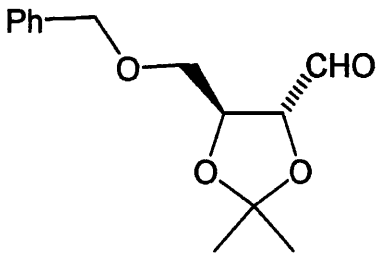

**(52)**




**(53)**

Sodium hydride (60% dispersion in mineral oil, 1.4 g, 34 mmol) was stirred in dry DMF (20 ml) under nitrogen for 30 min. A solution of (51) (5.0 g, 31 mmol) in DMF (20 ml) was then added dropwise and the reaction mixture was stirred for a further 30 min before benzyl chloride (4.0 g, 32 mmol) was added. The reaction mixture was stirred for 1.5 h, and was then poured into ice water (250 ml) and extracted with diethyl ether (3 × 150 ml). The combined extracts were washed successively with water and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The resulting oil was purified by chromatography (pet. ether (40-60 °C)/ether, 1:1) to afford (52) (2.2 g, 28%); $R_f$ = 0.79 as a yellow oil; NMR $\delta_H$ 1.42 (6H, s, $CMe_2$), 3.54-3.66 (4H, m, 2 × $CH_2$OBn), 4.00-4.04 (2H, m, 4,5-$H_2$), 4.54 (2H, d, $J$ = 12.3 Hz, 2 × CHPh), 4.58 (2H,d, $J$ = 12.3 Hz, 2 × CHPh), 7.3 (10H, m, 2 × Ph-$H_5$) and (53) (3.2 g, 64%) $R_f$ = 0.11 as a pale yellow oil. $[\alpha]^{20}_D$ = +8.0° (c 3.2, $CHCl_3$) (lit.[142] $[\alpha]^{23}_D$ = +8.3° (c 2.9, $CHCl_3$)); NMR $\delta_H$ 1.41 (3H, s, Me), 1.42 (3H, s, Me), 2.33 (1H, dd, $J$ = 8.6, 4.3 Hz, OH), 3.55 (1H, dd, $J$ = 9.8, 4.3 Hz, CHOBn), 3.64-3.70 (2H, m, C*H*OH + CHOBn), 3.75 (1H, dt, $J$ = 11.7, 4.3 Hz, C*H*OH), 3.94 (1H, dt, $J$ = 8.3, 4.3 Hz, 5-H), 4.05 (1H, dt, $J$ = 8.3, 4.3 Hz, 4-H), 4.58 (2H, s, $CH_2$Ph), 7.29-7.35 (5H, m, Ph-$H_5$).

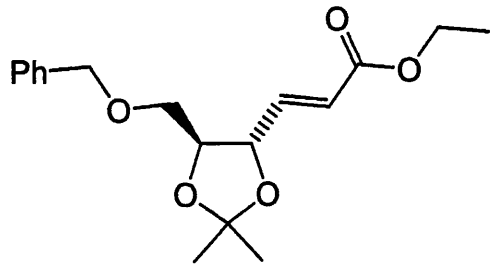
**(4*S*,5*R*)-4-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolane-5-carboxaldehyde (54)[142]**



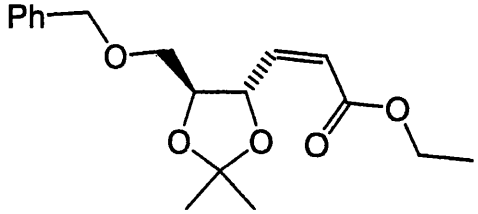
**(54)**

The alcohol (53) (3.6 g, 14 mmol) in dichloromethane (15 ml) was added slowly to a stirred slurry of pyridinium chlorochromate (3.6 g, 35 mmol), sodium acetate (0.30 g, 3.5 mmol) and powdered 4 Å molecular sieves (3 g) in dichloromethane (200 ml) under nitrogen. After stirring for 3 h the reaction mixture was filtered on silica gel with elution by ether. Evaporation of the solvent gave (54) (3.3 g, 93%) as a pale yellow oil. $[\alpha]^{20}_{D}$ = +14° (c 3, $CHCl_3$) (lit.[145] $[\alpha]^{21}_{D}$ = +16.8° (c 1.1, $CHCl_3$); NMR $\delta_H$ 1.43 (3H, s, Me), 1.50 (3H, s, Me), 3.67 (2H, d, $J$ = 4.0 Hz, $CH_2OBn$), 4.19-4.29 (2H, m, 4,5-$H_2$), 4.58 (1H, d, $J$ = 10.5 Hz, CHPh), 4.61 (1H, d, $J$ = 10.5 Hz, CHPh), 7.25-7.36 (5H, m, Ph-$H_5$), 9.76 (1H, d, $J$ = 1.5 Hz, CHO).

**(*E*,4*S*,5*S*)-4-Benzyloxymethyl-5-(ethoxycarbonylethenyl)-2,2-dimethyl-1,3-dioxolane (55) and (*Z*,4*S*,5*S*)-4-benzyloxymethyl-5-(ethoxycarbonylethenyl)-2,2-dimethyl-1,3-dioxolane (56)[142]**



**(55)**

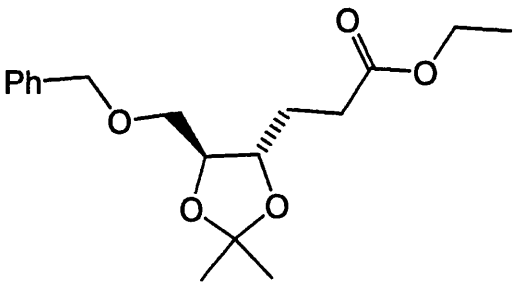


**(56)**

The aldehyde (54) (2 g, 8 mmol), ethyl triphenylphosphoranylideneacetate (4.2 g, 16 mmol) and benzoic acid (50 mg, 0.4 mmol) in toluene (200 ml) were heated at reflux under nitrogen for 4 h. The mixture was allowed to cool to room temperature before

the solvent was evaporated off under reduced pressure to give a white solid. This solid was extracted with diethyl ether (3 × 20 ml) and the ether was evaporated to give a pale yellow oil which gave, after chromatography (pet. ether (40-60 °C)/ether, 5:1), first *Z*-alkene (56) (0.8 g, 31%); $R_f$ = 0.31 as an oil and then the *E*-isomer (55) (0.80 g, 31%); $R_f$ = 0.18 also as an oil; $[\alpha]^{20}_D$ = -21° (c 4.5, $CHCl_3$) (lit.[142] $[\alpha]^{23}_D$ = -13.4° (c 2.1, $CHCl_3$). The *Z*-isomer (56) was characterised by NMR $\delta_H$ 1.25 (3H, t, *J* = 7.1 Hz, $CH_2CH_3$), 1.45 (6H, s, $CMe_2$), 3.68 (2H, d, *J* = 3.1 Hz, $CH_2OBn$), 3.94-4.00 (1H, m, 4-H), 4.12 (2H, q, *J* = 7.1 Hz, $CH_2CH_3$), 4.56 (1H, d, *J* = 12.1 Hz, CHPh), 4.62 (1H, d, *J* = 12.1 Hz, CHPh), 5.38 (1H, dt, *J* = 8.3, 1.2 Hz, 5-H), 5.92 (1H, dd, *J* = 11.7, 1.2 Hz, $CHCO_2$), 6.18 (1H, dd, *J* = 11.7, 8.3 Hz, CH=CH), 7.32-7.37 (5H, m, Ph-$H_5$). The *E*-isomer had NMR $\delta_H$ 1.29 (3H, t, *J* = 7.0 Hz, $CH_2CH_3$), 1.43 (3H, s, Me), 1.45 (3H, s, Me), 3.62 (2H, d, *J* = 4.7 Hz, $CH_2OBn$), 3.95 (1H, dt, *J* = 8.6, 4.7 Hz, 4-H), 4.19 (2H, q, *J* = 7.0 Hz, $CH_2CH_3$), 4.42 (1H, ddd, *J* = 8.6, 5.5, 1.4 Hz, 5-H), 4.56 (1H, *J* = 12.1 Hz, CHPh), 4.61 (1H, d, *J* = 12.1 Hz, CHPh), 6.09 (1H, dd, *J* = 15.6, 1.4 Hz, $CHCO_2$), 6.88 (1H, dd, *J* = 15.6, 5.5 Hz, CH=CH), 7.27-7.36 (5H, m, Ph-$H_5$).

**(4*S*,5*S*)-4-Benzyloxymethyl-5-(ethoxycarbonylethyl)-2,2-dimethyl-1,3-dioxolane (57)[142]**

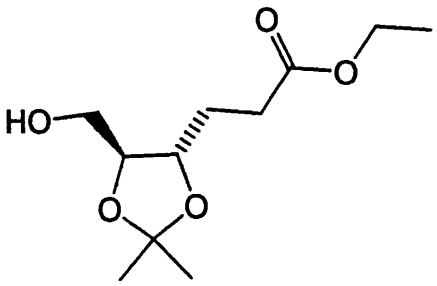



**(57)**

A mixture of the unsaturated esters (55) and (56) (0.62 g, 1.9 mmol) was stirred in ethanol (25 ml) with Pd/C (5%, 30 mg) under atmosphere of hydrogen for 1 h. The catalyst was removed by filtration through Celite®. The solvent was evaporated off and the residue was purified by chromatography (pet. ether (40-60 °C)/ether, 4:1) to give (57) (0.4 g, 63%); $R_f$ = 0.29 as a pale yellow oil. $[\alpha]^{20}_D$ = -15° (c 4.0, $CHCl_3$); NMR $\delta_H$ 1.23 (3H, t, *J* = 7.0 Hz, $CH_2CH_3$), 1.38 (3H, s, Me), 1.39 (3H, s, Me), 1.81-1.87 (1H, m, $CHCH_2$), 1.93-1.99 (1H, m, $CHCH_2$), 2.37-2.54 (2H, m, $CH_2CO_2$),

3.53-3.60 (2H, m, $CH_2OBn$), 3.80-3.87 (2H, m, 4,5-$H_2$), 4.12 (2H, q, $J$ = 7.0 Hz, $CH_2CH_3$), 4.56 (1H, d, $J$ = 12.3 Hz, CHPh), 4.59 (1H, d, $J$ = 12.3 Hz, CHPh), 7.32-7.34 (5H, m, Ph-$H_5$); MS *m/z* 323.1856 (M + H) ($C_{19}H_{26}O_5$ requires 323.1858), 265 (M – $C_3H_5O$), 91 (Bn).

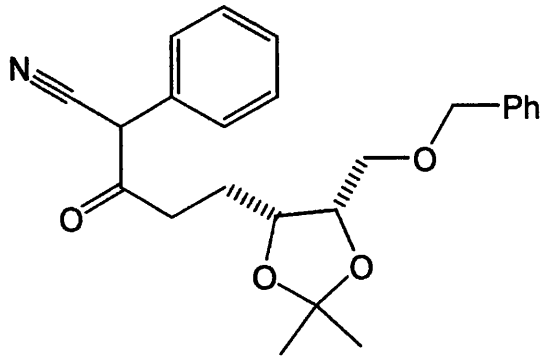
**(4*S*,5*S*)-5-(Ethoxycarbonylethyl)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (58)[142]**




**(58)**

A mixture of the unsaturated esters (55) and (56) (0.66 g, 2.1 mmol) was stirred in ethanol (20 ml) with Pd/C (5%, 35 mg) under an atmosphere of hydrogen for 6h. The catalyst was removed by filtration through Celite®. The solvent was evaporated to afford (58) (0.45 g, 93%); as a pale yellow oil; NMR $\delta_H$ 1.26 (3H, t, $J$ = 7.0 Hz, $CH_2CH_3$), 1.39 (3H, s, Me), 1.40 (3H, s, Me), 1.79-1.89 (1H, m, $CHCH_2$), 1.92-2.01 (1H, m, $CHCH_2$), 2.39-2.55 (3H, m, $CH_2CO_2$ + OH), 3.60 (1H, dd, $J$ = 11.5, 4.1 Hz, $CHOH$), 3.70-3.82 (2H, m, $CHOH$ + 4-H), 3.91 (1H, dt, $J$ = 8.2, 3.8 Hz, 5-H), 4.13 (2H, q, $J$ = 7.0 Hz, $CH_2CH_3$).

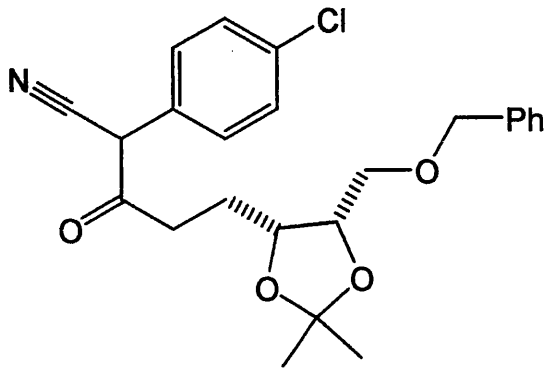
**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-oxo-4-phenylbutyl)-2,2-dimethyl-1,3-dioxolane (61)**




**(61)**

Lithium bis(trimethylsilyl)amide (1 M in THF) (9.1 ml, 9.1 mmol) was added to a stirred solution of phenylacetonitrile (1.1 g, 9.1 mmol) in dry diethyl ether (10 ml) under nitrogen at -78 °C. After 10 min, compound (48) (2.9 g, 9.1 mmol) was added and the reaction mixture was allowed to warm to room temperature. The mixture was stirred for 72 h. An excess of diethyl ether was added, followed by water. The aqueous layer was separated, washed with diethyl ether and then added to ethyl acetate (100 ml) in a separating funnel. Acidification by dropwise addition of 1 M aqueous citric acid to pH = 6 with rapid extraction with ethyl acetate was followed by rapid separation of the organic layer. Ethyl acetate was washed with water, dried ($MgSO_4$) and evaporated. Purification with chromatography (ethyl acetate/hexane, 2:1) afforded (61) (1.5 g, 21%); $R_f$ = 0.34 as a pale yellow oil; IR $\nu_{max}$ 2207 (CN), 1728 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.26 (3H, s, Me), 1.34 (3H, s, Me), 1.66-1.80 (2H, m, C*H*$_2$CHO), 2.58-2.68 (1H, m, C*H*CH$_2$), 2.70-2.80 (1H, m, C*H*CH$_2$), 3.45 (2H, d, *J* = 6.0 Hz, $CH_2OBn$), 3.95-4.02 (1H, m, 4-H), 4.20 (1H, q, *J* = 6.0 Hz, 5-H), 4.45 (1H, d, *J* = 11.5 Hz, CHPh), 4.52 (1H, d, *J* = 11.5 Hz, CHPh), 7.21-7.41 (10H, m, 2 × Ph-H$_5$), 8.96 (1H, s, OH); MS *m/z* 394.2016 (M + H) ($C_{24}H_{28}NO_4$ requires 394.2018), 336 (M - $C_2H_3NO$), 91 (Bn).

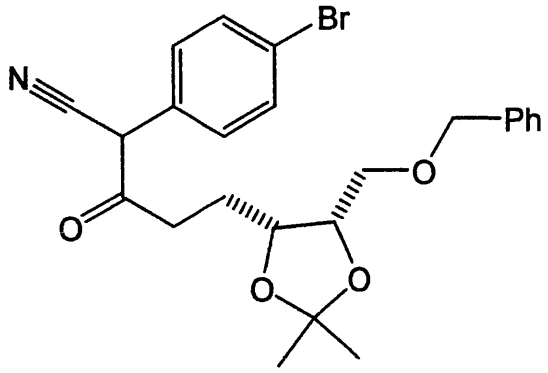
**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (62)**




**(62)**

4-Chlorophenylacetonitrile (3.0 g, 20 mmol) and compound (48) (6.4 g, 20 mmol) were condensed in the same method as described above. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 1:1) to afford (62) (2.2 g, 26%); $R_f$ = 0.58 as a pale yellow oil; NMR $\delta_H$ 1.40 (3H, s, Me), 1.51 (3H, s, Me), 1.78-1.88 (2H, m, C$H_2$CHO), 1.98-2.08 (2H, m, C$H_2$CH$_2$), 3.45 (2H, d, $J$ = 6.0 Hz, CH$_2$OBn), 4.29 (1H, ddd, $J$ = 10.1, 6.0, 3.9 Hz, 4-H), 4.39 (1H, q, $J$ = 6.0 Hz, 5-H), 4.45 (1H, d, $J$ = 11.5 Hz, CHPh), 4.49 (1H, d, $J$ = 11.5 Hz, CHPh), 5.52 (0.1H, s, CHCN), 7.16 (0.2H, d, $J$ = 8.6 Hz, Ph 3,5-H$_2$), 7.24 (1.8H, d, $J$ = 8.6 Hz, Ph 3,5-H$_2$), 7.25-7.32 (5H, m, Ph-H$_5$), 7.35 (1.8H, d, $J$ = 8.6 Hz, Ph 2,6-H$_2$), 7.84 (0.2H, d, $J$ = 8.6 Hz, Ph 2,6-H$_2$), 9.33 (0.9H, br, OH); MS *m/z* 430.1604 (M + H) ($C_{24}H_{26}{}^{37}ClNO_4$ requires 430.1599), 428.1618 (M + H) ($C_{24}H_{27}{}^{35}ClNO_4$ requires 428.1628), 372/370 (M - $C_2H_4NO$), 91 (Bn).

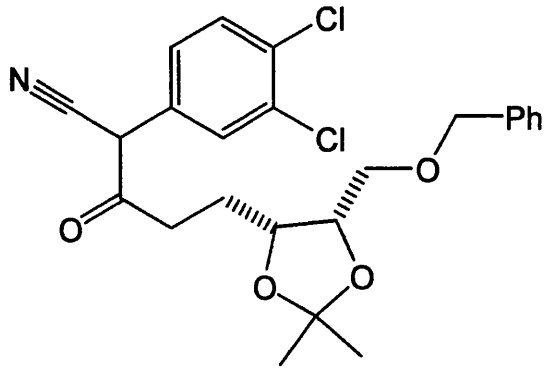
**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (63)**




**(63)**

4-Bromophenylacetonitrile (3.9 g, 20 mmol) and compound (48) (6.4 g, 20 mmol) were condensed in the same method as described above. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (63) (1.7 g, 18%); $R_f$ = 0.82 as a yellow oil; NMR $\delta_H$ 1.27 (3H, s, Me), 1.35 (3H, s, Me), 1.70-1.82 (2H, m, C$H_2$CHO), 2.62-2.78 (2H, m, $CH_2$C=O), 3.45-3.47 (2H, m, $CH_2$OBn), 4.00 (1H, ddd, *J* = 10.1, 6.2, 3.9 Hz, 4-H), 4.22 (1H, q, *J* = 6.2 Hz, 5-H), 4.45 (1H, d, *J* = 11.9 Hz, CHPh), 4.49 (1H, d, *J* = 11.9 Hz, CHPh), 5.48 (0.35H, s, CHCN), 7.21-7.35 (5H, m, Ph-$H_5$), 7.45 (1.35H, d, *J* = 8.8 Hz, Ph 3,5-$H_2$), 7.55 (1.35H, d, *J* = 8.8 Hz, Ph 2,6-$H_2$), 7.59 (0.65H, d, *J* = 8.6 Hz, Ph 3,5-$H_2$), 7.76 (0.65H, d, *J* = 8.8 Hz, Ph 2,6-$H_2$), 9.35 (0.65H, s, OH); MS *m/z* 474.1100 (M + H) ($C_{24}H_{27}{}^{81}BrNO_4$ requires 474.1102), 472.1094 (M + H) ($C_{24}H_{27}{}^{79}BrNO_4$ requires 472.1123), 415/413 (M - $C_2H_4NO$), 91 (Bn).

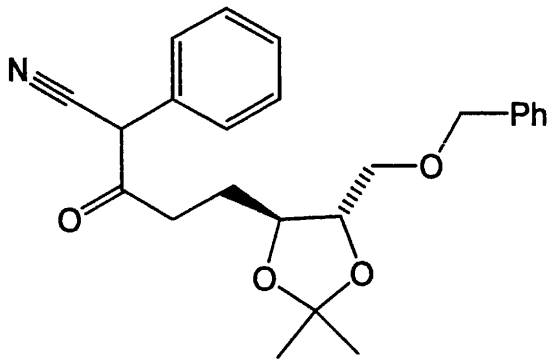
**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(3,5-dichlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (64)**




**(64)**

3,4-Dichlorophenylacetonitrile (3.7 g, 20 mmol) and compound (48) (6.4 g, 20 mmol) were condensed in the same method as described above. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (64) (1.5 g, 16%); $R_f$ = 0.68 as a hygroscopic white solid; NMR $\delta_H$ 1.41 (3H, s, Me), 1.52 (3H, s, Me), 1.73-1.87 (2H, m, C*H*$_2$CHO), 2.71-2.84 (2H, m, $CH_2C$=O), 3.47 (1H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.49 (1H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.90-4.07 (1H, m, 4-H), 4.22 (1H, q, *J* = 6.0 Hz, 5-H), 4.47 (1H, d, *J* = 12.3 Hz, CHPh), 4.56 (1H, d, *J* = 12.3 Hz, CHPh), 7.26-7.37 (5H, m, Ph-$H_5$), 7.45 (1H, d, *J* = 8.6 Hz, Ph 5-H), 7.50 (1H, dd, *J* = 8.6, 2.0 Hz, Ph 6-H), 7.83 (1H, d, *J* = 2.0 Hz, Ph 2-H), 9.61 (1H, br, OH); MS *m/z* 466.1178 (M + H) ($C_{24}H_{26}{}^{37}Cl_2NO_4$ requires 466.1179), 464.1193 (M + H) ($C_{24}H_{26}{}^{37}Cl^{35}ClNO_4$ requires 464.1209), 462.1217 (M + H) ($C_{24}H_{26}{}^{35}Cl_2NO_4$ requires 462.1238), 409/407/405/403 (M - $C_2H_4NO$), 91 (Bn).

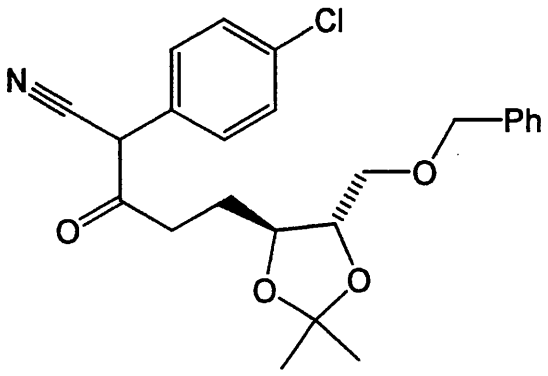
**(4*S*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-oxo-4-phenylbutyl)-2,2-dimethyl-1,3-dioxolane (65)**




**(65)**

Lithium bis(trimethylsilyl)amide (1.0 M in THF) (1.3 ml, 1.3 mmol) was added to a stirred solution of phenylacetonitrile (0.14 g, 1.3 mmol) in dry diethyl ether (5 ml) under nitrogen at -78 °C. After 10 min, compound (57) (0.40 g, 1.3 mmol) was added and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred for 72 h. An excess of diethyl ether was added, followed by water. The aqueous layer was separated, washed with diethyl ether and then acidified by dropwise addition of 1 M aqueous citric acid to pH = 6. The aqueous layer was extracted with diethyl ether (3 × 50ml) and the combined ether extracts was washed with water, dried ($MgSO_4$) and evaporated. Purification by chromatography (ethyl acetate/hexane, 2:1) afforded (65) (70 mg, 14%); $R_f$ = 0.45 as a pale yellow solid: mp 75-77 °C; IR $\nu_{max}$ 2206 (CN), 1731 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.39 (3H, s, Me), 1.41 (3H, s, Me), 1.82-1.90 (1H, m, C*H*CHO), 1.97-2.03 (1H, m, C*H*CHO), 2.48-2.65 (2H, m, C$H_2$CO), 3.54-3.63 (2H, m, 4,5-$H_2$), 3.87-3.89 (2H, m, $CH_2$OBn), 4.57 (1H, d, *J* = 12.5 Hz, CHPh), 4.61 (1H, d, *J* = 12.5 Hz, CHPh), 5.59 (0.2H, s, CHCN), 7.33-7.64 (8H, m, Ph 3,4,5-$H_3$ + Ph-$H_5$), 7.66 (1.6H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$), 7.91 (0.4H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$), 9.19 (0.8H, br, OH); MS *m/z* 392.1859 (M - H) ($C_{24}H_{26}NO_4$ requires 392.1861), 335 (M - $C_2H_4NO$), 317 (M – $C_7H_6$), 91 (Bn).

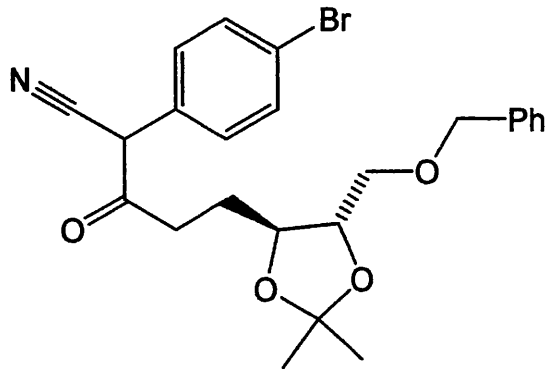
**(4*S*,5*S*)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (66)**




**(66)**

4-Chlorophenylacetonitrile (0.19 g, 1.3 mmol) and compound (57) (0.4 g, 1.3 mmol) were condensed in the same method as described above. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 1:1) to afford (66) (0.22 g, 41%); $R_f$ = 0.68 as a yellow oil; IR $\nu_{max}$ 2209 (CN), 1731 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.39 (3H, s, Me), 1.40 (3H, s, Me), 1.81-1.88 (1H, m, C*H*CHO), 1.95-2.04 (1H, m, C*H*CHO), 2.46-2.63 (2H, m, C$H_2$CO), 3.53-3.62 (2H, m, 4,5-$H_2$), 3.83-3.87 (2H, m, $CH_2$OBn), 4.57 (1H, d, $J$ = 12.1 Hz, CHPh), 4.60 (1H, d, $J$ = 12.1 Hz, CHPh), 7.26-7.40 (5H, m, Ph-$H_5$), 7.44 (2H, d, $J$ = 8.6 Hz, Ph 3,5-$H_2$), 8.02 (2H, d, $J$ = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 430.1608 (M + H) ($C_{24}H_{27}{}^{37}ClNO_4$ requires 430.1599), 428.1623 (M + H) ($C_{24}H_{27}{}^{35}ClNO_4$ requires 428.1628), 372/370 (M – $C_2H_3NO$), 91 (Bn).

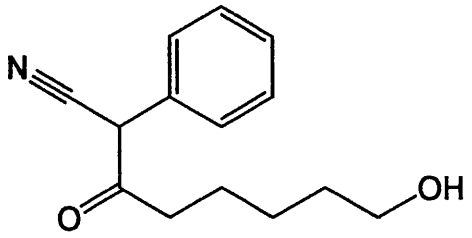
**(4*S*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-oxobutyl)-2,2-dimethyl-1,3-dioxolane (67)**




**(67)**

4-Bromophenylacetonitrile (2.9 g, 15 mmol) and compound (57) (4.8 g, 15 mmol) were condensed in the same method as described above. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (67) (1.7 g, 24%); $R_f$ = 0.81 as a pale yellow oil; IR $\nu_{max}$ 2208 (CN), 1718 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.33 (3H, s, CMe), 1.34 (3H, s, CMe), 1.68-1.81 (1H, m, C*H*CHO), 1.83-1.98 (1H, m, C*H*CHO), 2.66-2.72 (1H, m, CHCO), 2.74-2.84 (1H, m, CHCO), 3.55-3.65 (2H, m, $CH_2$OBn), 3.91-3.95 (1H, m, 5-H), 4.03 (1H, dt, *J* = 8.0, 3.7 Hz, 4-H), 4.53 (1H, d, *J* = 12.0 Hz, CHPh), 4.61 (1H, d, *J* = 12.0 Hz, CHPh),5.50 (0.25H, s, CHCN), 7.22 (1.5H, d, *J* = 8.1 Hz, Ph 3,5-$H_2$), 7.26-7.36 (5H, m, Ph-$H_5$), 7.53 (1.5H, d, *J* = 8.1 Hz, Ph 2,6-$H_2$), 7.60 (0.5H, d, *J* = 8.7 Hz, Ph 3,5-$H_2$), 7.77 (0.5H, d, *J* = 8.7 Hz, Ph 2,6-$H_2$), 9.60 (0.75H, br, OH); MS *m/z* 474.1103 (M + H) ($C_{24}H_{27}{}^{81}BrNO_4$ requires 474.1102), 472.1103 (M + H) ($C_{24}H_{27}{}^{79}BrNO_4$ requires 472.1123), 415/413 (M – $C_2H_4NO$), 91 (Bn).

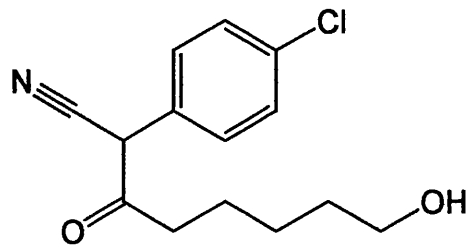
**1-Cyano-7-hydroxy-1-phenylheptan-2-one (68)**




**(68)**

Phenylacetonitrile (1.2 g, 10.2 mmol) and ε-caprolactone (1.2 g, 10 mmol) were condensed in the same method described for compound (65). The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (68) (0.50 g, 21%); $R_f$ = 0.26 as a pale yellow solid: mp 98-99 °C; IR $\nu_{max}$ 3402 (OH), 2205 (CN), 1718 (C=O) $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.35-1.42 (2H, m, 5-$H_2$), 1.44-1.51 (2H, m, 6-$H_2$), 1.65 (2H, qn, $J$ = 7.4 Hz, 4-$H_2$), 2.60 (2H, t, $J$ = 7.4 Hz, 3-H), 3.40 (2H, t, $J$ = 6.2 Hz, 7-$H_2$), 4.36 (1H, s, OH), 7.20 (1H, t, $J$ = 7.6 Hz, Ph 4-H), 7.30 (2H, t, $J$ = 7.6 Hz, Ph 3,5-$H_2$), 7.61 (2H, d, $J$ = 7.6 Hz, Ph 2,6-$H_2$), 11.52 (1H, br, OH); MS *m/z* 232.1329 (M + H) ($C_{14}H_{18}NO_2$ requires 232.1337), 214 (M - OH), 185 (M – $C_2H_6O$), 115 (M – $C_6H_{12}O_2$).

**1-(4-Chlorophenyl)-1-cyano-7-hydroxy-1-phenylheptan-2-one (69)**

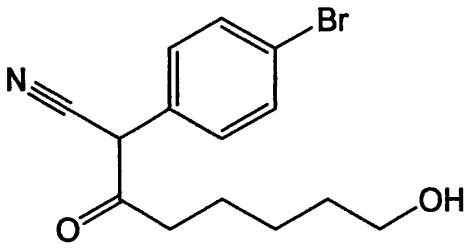



**(69)**

4-Chlorophenylacetonitrile (1.5 g, 10 mmol) and ε-caprolactone (1.1 g, 10 mmol) were condensed in the same method described for compound (65). The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (69) (0.30 g, 11%); $R_f$ = 0.16 as a white solid: mp 92-94 °C; NMR $\delta_H$ 1.21-1.30 (2H, m,

5-$H_2$), 1.50 (2H, m, 6-$H_2$), 1.58 (2H, qn, $J$ = 7.4 Hz, 4-$H_2$), 2.58 (1H, dt, $J$ = 18.2, 7.4 Hz, 3-H), 2.66 (1H, dt, $J$ = 18.2, 7.4 Hz, 3-H), 3.60 (2H, t, $J$ = 6.8 Hz, 7-$H_2$), 4.65 (1H, s, CHCN), 7.32 (2H, d, $J$ = 8.4 Hz, Ph 3,5-$H_2$), 7.41 (2H, d, $J$ = 8.4 Hz, Ph 2,6-$H_2$); MS *m/z* 268.0912 (M + H) ($C_{14}H_{17}{}^{37}ClNO_2$ requires 268.0918), 266.0942 (M + H) ($C_{14}H_{17}{}^{35}ClNO_2$ requires 266.0947), 250/248 (M – OH), 207/205 (M - $C_3H_8O$).

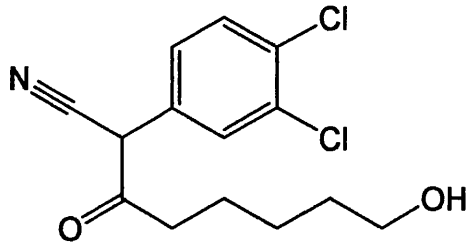
**1-(4-Bromophenyl)-1-cyano-7-hydroxy-1-phenylheptan-2-one (70)**




**(70)**

4-Bromophenylacetonitrile (1.3 g, 6.6 mmol) and ε-caprolactone (0.73 g, 6.6 mmol) were condensed in the same method described for compound (65). The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (70) (0.3 g, 15%); $R_f$ = 0.24 as a pale yellow solid: mp 76-78 °C; NMR $\delta_H$ 1.28 (2H, qn, $J$ = 7.3 Hz, 5-$H_2$), 1.50 (2H, m, 6-$H_2$), 1.58 (2H, qn, $J$ = 7.3 Hz, 4-$H_2$), 2.62 (1H, dt, $J$ = 18.0, 7.3 Hz, 3-H), 2.65 (1H, dt, $J$ = 18.0, 7.3 Hz, 3-H), 3.60 (2H, t, $J$ = 6.4 Hz, 7-$H_2$), 4.64 (1H, s, CHCN), 7.26 (2H, d, $J$ = 8.2 Hz, Ph 3,5-$H_2$), 7.41 (2H, d, $J$ = 8.2 Hz, Ph 2,6-$H_2$); MS *m/z* 312.0430 (M + H) ($C_{14}H_{17}{}^{81}ClNO_2$ requires 312.0422), 310.0449 (M + H) ($C_{14}H_{17}{}^{79}ClNO_2$ requires 310.0442), 294/292 (M – OH).

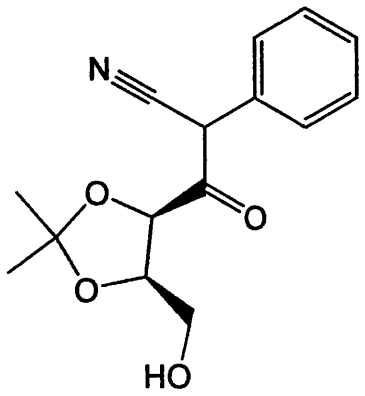
**1-(3,4-Dichlorophenyl)-1-cyano-7-hydroxy-1-phenylheptan-2-one (71)**




**(71)**

3,4-Dichlorophenylacetonitrile (1.4 g, 7.6 mmol) and ε-caprolactone (0.84 g, 7.6 mmol) were condensed in the same method described for compound (65). The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (71) (0.80 g, 35%); $R_f$ = 0.39 as a pale yellow solid: mp 95-97 °C; NMR $\delta_H$ 1.26 (2H, qn, *J* = 7.3 Hz, 5-$H_2$), 1.53 (2H, m, 6-$H_2$), 1.62 (2H, qn, *J* = 7.3 Hz, 4-$H_2$), 2.66 (2H, m, 3-$H_2$), 3.60 (2H, t, *J* = 6.4 Hz, 7-$H_2$), 4.69 (1H, s, CHCN), 7.39 (1H, d, *J* = 8.4 Hz, Ph 6-H), 7.51 (1H, d, *J* = 8.4 Hz, Ph 5-H), 7.83 (1H, s, Ph 2-H); MS *m/z* 304.0520 (M + H) ($C_{14}H_{16}{}^{37}Cl_2NO_2$ requires 304.0499), 302.0537 (M + H) ($C_{14}H_{16}{}^{37}Cl^{35}ClNO_2$ requires 302.0528), 300.0559 (M + H) ($C_{14}H_{16}{}^{35}Cl_2NO_2$ requires 300.0558), 286/284/282 (M – OH), 215/213/211 (M – $C_4H_9NO$), 179 (M – $C_4H_9ClNO$).

**(4*R*,5*R*)-4-(2-Cyano-1-oxo-2-phenylethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (72)**

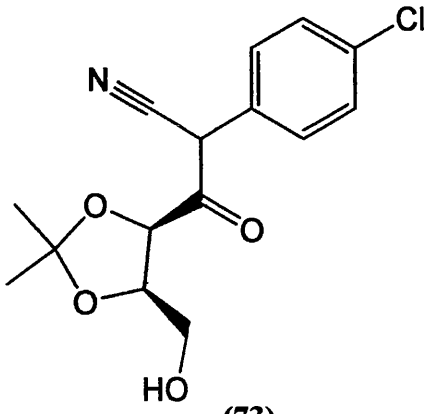



**(72)**

Phenylacetonitrile (0.50 g, 4.4 mmol) was condensed with 2,3-O-isopropylidene–D-erythronolactone (0.70 g, 4.4 mmol) in the same manner as described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to give 72) (0.30 g, 27%); $R_f$ = 0.81 as a pale yellow oil; IR $\nu_{max}$ 3408 (OH), 2246 (CN), 1694 (CO) $cm^{-1}$; NMR $\delta_H$ 1.41 (3H, s, Me), 1.49 (3H, s, Me), 4.41 (1H, dd, *J* = 11.0, 3.5 Hz, C*H*OH), 4.48 (1H, d, *J* = 11.0 Hz, C*H*OH), 4.75 (1H, d, *J* = 5.5 Hz, 4-H), 4.87-4.89 (1H, m, 5-H), 7.47 (2H, t, *J* = 7.4 Hz, Ph 3,5-$H_2$), 7.61 (1H, t, *J* = 7.4 Hz, Ph 4-H), 8.10 (2H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 276.1225 (M + H)

($C_{15}H_{18}NO_4$ requires 276.1235), 258 (M – OH), 243 (M - $CH_4O$).

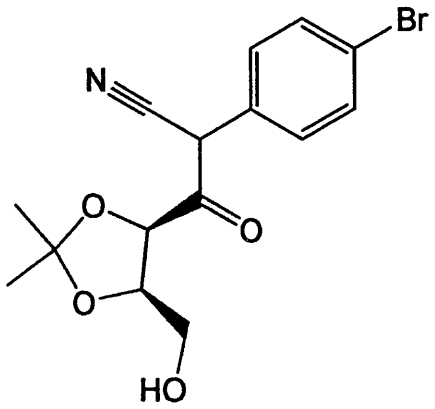
**(4*R*,5*R*)-4-(2-(4-Chlorophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (73)**




**(73)**

4-Chlorophenylacetonitrile (0.80 g, 5 mmol) was condensed with 2,3-O-isopropylidene–D-erythronolactone (0.80 g, 5 mmol) in the same manner as described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to give (73) (0.33 g, 21%); $R_f$ = 0.79 as a pale yellow oil; NMR $\delta_H$ 1.30 (3H, s, Me), 1.59 (3H, s, Me), 4.05 (1H, dd, *J* = 10.1, 3.4 Hz, C*H*OH), 4.09 (1H, d, *J* = 10.1 Hz, C*H*OH), 4.73 (1H, d, *J* = 5.8 Hz, 4-H), 4.92 (1H, dd, *J* = 5.8, 3.4 Hz, 5-H), 7.27 (2H, d, *J* = 8.4 Hz, Ph 3,5-$H_2$), 7.48 (2H, d, *J* = 8.4 Hz, Ph 2,6-$H_2$); MS *m/z* 312.0849 (M + H) ($C_{15}H_{17}{}^{37}ClNO_4$ requires 312.0816), 310.0855 (M + H) ($C_{15}H_{16}{}^{35}ClNO_4$ requires 310.0846), 294/292 (M –OH), 254/252 (M – $C_2H_3NO_4$).

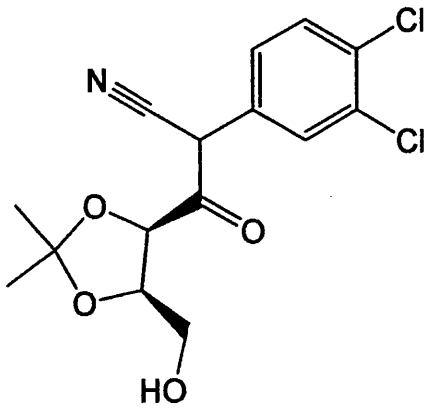
**(4*R*,5*R*)-4-(2-(4-Bromophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (74)**




**(74)**

4-Bromophenylacetonitrile (3.0 g, 15 mmol) was condensed with 2,3-O-isopropylidene–D-erythronolactone (2.4 g, 15 mmol) in the same manner as described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 3:1) to give (74) (2 g, 38%); $R_f$ = 0.89 as a pale yellow oil; IR $\nu_{max}$ 3422 (OH), 2208 (CN), 1777 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.39 (3H, s, Me), 1.47 (3H, s, Me), 4.40 (1H, dd, *J* = 10.9, 3.7 Hz, C*H*OH), 4.45 (1H, d, *J* = 10.9 Hz, *CH*OH), 4.74 (1H, d, *J* = 5.5 Hz, 4-H), 4.86-4.88 (1H, m, 5-H), 5.57 (0.35H, s, CHCN), 7.27 (1.3H, d, *J* = 8.6 Hz, Ph 3,5-$H_2$), 7.51 (1.3H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$), 7.59 (0.7H, d, *J* = 8.6 Hz, Ph 3,5-$H_2$), 7.78 (0.7H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 356.0326 (M + H) ($C_{15}H_{17}{}^{81}BrNO_4$ requires 356.0320), 354.0327 (M + H) ($C_{15}H_{17}{}^{79}BrNO_4$ requires 354.0340), 338/336 (M – OH), 272 (M – HBr).

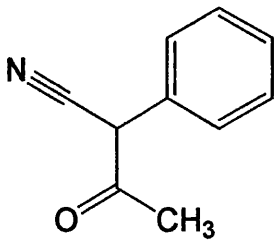
**(4*R*,5*R*)-4-(2-(3,4-Dichlorophenyl)-2-cyano-1-oxoethyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (75)**




**(75)**

3,4-Dichlorophenylacetonitrile (3.7 g, 20 mmol) was condensed with 2,3-O-isopropylidene–D-erythronolactone (3.2 g, 20 mmol) in the same manner as described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 1:1) to give (75) (1.5 g, 22%); $R_f$ = 0.81 as a pale yellow oil; IR $\nu_{max}$ 3404 (OH), 2250 (CN), 1782 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.30 (3H, s, Me), 1.38 (3H, s, Me), 3.93 (1H, dd, *J* = 10.3, 3.7 Hz, C*H*OH), 4.00 (1H, d, *J* = 10.3 Hz, C*H*OH), 4.70 (1H, d, *J* = 5.9 Hz, 4-H), 4.91 (1H, dd, *J* = 5.9, 3.7 Hz, 5-H), 7.33 (1H, dd, *J* = 8.2, 2.0 Hz, Ph 6-H), 7.36 (1H, d, *J* = 8.2 Hz, Ph 5-H), 7.58 (1H, d, *J* = 2.0 Hz, Ph 2-H); MS *m/z* 348.0411 (M + H) ($C_{15}H_{16}{}^{37}Cl_2NO_4$ requires 348.0397), 346.0901 (M + H) ($C_{15}H_{16}{}^{37}Cl^{35}ClNO_4$ requires 346.0906), 344.0448 (M + H) ($C_{15}H_{16}{}^{35}Cl_2NO_4$ requires 344.0456), 330/328/326 (M – OH), 288/286/284 (M - $C_2H_4NO$).

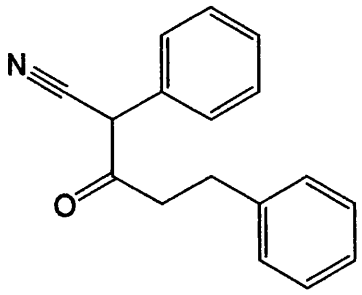
**1-Cyano-1-phenylpropan-2-one (76)**




**(76)**

Phenylacetonitrile (1.80 g, 15 mmol) and ethyl acetate (1.30 g, 15 mmol) were condensed by the same method as described before. An excess of diethyl ether was then added, followed by water. The aqueous layer was acidified with 1M aqueous HCl and the resulting precipitate was filtered and recrystallised from aqueous ethanol to afford (76) (0.75 g, 31%) as a buff solid: mp 87-88 °C (lit.[112] mp 87-89 °C); NMR $\delta_H$ 2.25 (1H, s, $CH_3$), 4.66 (1H, s, CHCN), 7.38-7.47 (5H, m, Ph-$H_5$); MS *m/z* 160.0740 (M + H) ($C_{10}H_{10}NO$ requires 160.0762), 144 (M - $CH_3$), 118 (M – $C_2H_3N$).

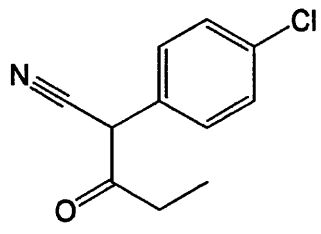
**1-Cyano-1,4-diphenylbutan-2-one (77)**




**(77)**

Phenylacetonitrile (1.75 g, 15 mmol) and ethyl 3-phenylpropanoate (2.70 g, 15 mmol) were condensed by the same method as described above to afford (77) (1.20 g, 34%) as a buff solid: mp 53-54 °C; IR $\nu_{max}$ 2200 (CN) $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 2.88 (2H, t, $J$ = 8.0 Hz, $CH_2$), 2.94 (2H, t, $J$ = 8.0 Hz, $CH_2$), 7.20-7.61 (10H, m, 2 × Ph-$H_5$), 11.70 (1H, br, OH); MS *m/z* 250.1240 (M + H) ($C_{17}H_{16}NO$ requires 250.1231), 222 (M – CHN), 91 (Bn).

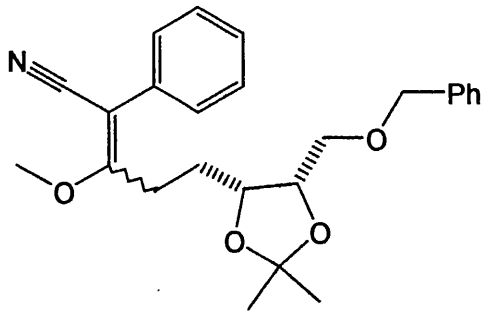
**1-(4-Chlorophenyl)-1-cyanobutan-2-one (78)**



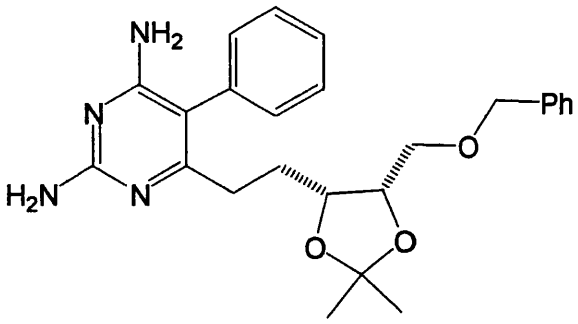

**(78)**

4-Chlorophenylacetonitrile (3.03 g, 20 mmol) and ethyl propionate (2.04 g, 20 mmol) were condensed by the same method as described above to afford (78) (1.55 g, 37%) as a pale yellow solid: mp 50-51 °C (lit.[110] mp 50-52 °C); NMR ($(CD_3)_2SO$) $\delta_H$ 1.24 (3H, t, $J$ = 7.4 Hz, $CH_3$), 2.62 (2H, q, $J$ = 7.4 Hz, $CH_2$), 7.42 (2H, d, $J$ = 8.8 Hz, 2,6-$H_2$), 7.66 (2H, d, $J$ = 8.8 Hz, 3,5-$H_2$), 11.87 (1H, br, OH) (enol form).

**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (82) and 2,4-diamino-6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine (100)**




(82)

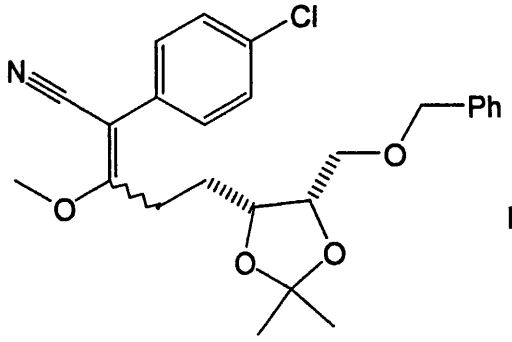



(100)

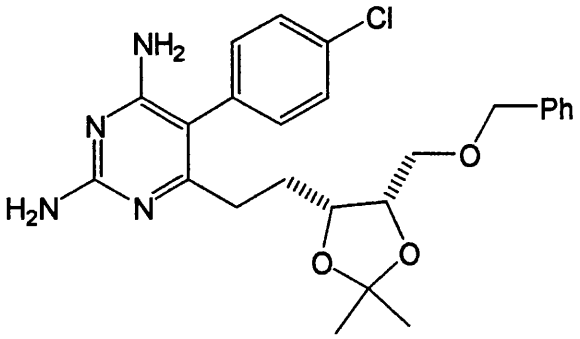
A solution of compound (61) (1.5 g, 3.7 mmol) in THF (5 ml) was treated with a solution of diazomethane (~0.3 g, 8 mmol) prepared from N-methyl-N-nitroso-4-toluenesulfonamide and KOH (using Minidiazald apparatus) in diethyl ether (20 ml) and the mixture was kept overnight at 10 °C. The excess of diazomethane was destroyed by dropwise addition of acetic acid (30% in THF), and the solvent was evaporated off to afford (82) (1.2 g, 81%) as a pale yellow oil; NMR $\delta_H$ 1.37 (3H, s, Me), 1.46 (3H, s, Me), 1.77-1.88 (2H, m, C$H_2$CHO), 2.78-2.86 (1H, m, C$H$CH$_2$), 2.90-2.98 (1H, m, C$H$CH$_2$), 3.53 (2H, d, $J$ = 5.9 Hz, $CH_2$OBn), 3.78 (3H, s, $OCH_3$), 4.18-4.25 (1H, m, 4-H), 4.31-4.34 (1H, m, 5-H), 4.51 (1H, d, $J$ = 12.1 Hz, CHPh), 4.59 (1H, d, $J$ = 12.1 Hz, CHPh), 7.21-7.59 (10H, m, 2 × Ph-$H_5$); MS *m/z* 408.2166 (M + H) ($C_{25}H_{30}NO_4$ requires 408.2174), 350 (M – $C_2H_3NO$), 91 (Bn). A solution of sodium methoxide (0.14 g, 2.6 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.3 g, 2.6 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The

alcoholic guanidine solution was boiled under reflux with compound (82) (0.7 g, 1.8 mmol) overnight. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9.5:0.5) to afford (100) (0.4 g, 46%); $R_f$ = 0.45 as a hygroscopic pale yellow solid; IR $\nu_{max}$ 3415 ($NH_2$), 1685 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.23 (3H, s, Me), 1.24 (3H, s, Me), 1.61-1.67 (2H, m, C*H*$_2$CHO), 2.20-2.27 (1H, m, C*H*CH$_2$), 2.48-2.55 (1H, m, C*H*CH$_2$), 3.4 (2H, d, *J* = 6.0 Hz, $CH_2OBn$), 3.95-4.00 (1H, m, 4-H), 4.15 (1H, q, *J* = 6.0 Hz, 5-H), 4.44 (1H, d, *J* = 12.3 Hz, CHPh), 4.53 (1H, d, *J* = 12.3 Hz, CHPh), 4.68 (2H, br, $NH_2$), 5.01 (2H, br, $NH_2$), 7.09-7.39 (10H, m, 2 × Ph-$H_5$); MS *m/z* 435.2423 (M + H) ($C_{25}H_{31}N_4O_3$ requires 435.2396), 200 (M – $C_{14}H_{18}O_3$), 91 (Bn).

**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (83) and 2,4-diamino-6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4yl)ethyl)-5-(4-chlorophenyl)pyrimidine (101)**




(83)

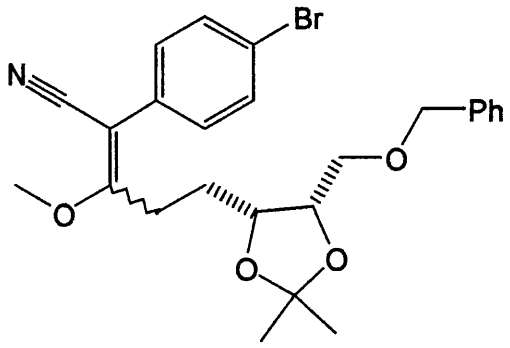



(101)

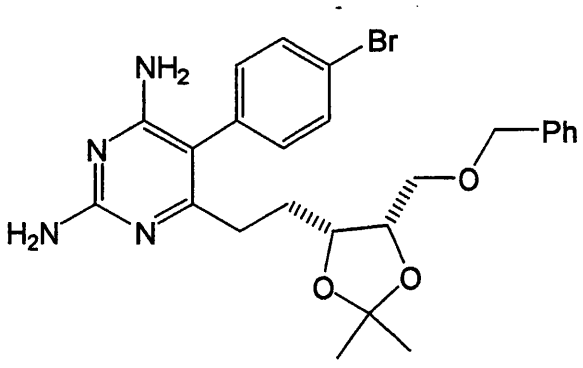
A solution of compound (62) (2.2 g, 5.2 mmol) in THF (5 ml) was methylated with diazomethane in the same method described above to afford (83) (2.2 g, 97%) as a pale yellow oil; NMR $\delta_H$ 1.36 (3H, s, Me), 1.45 (3H, s, Me), 1.72-1.79 (1H, m, C*H*CHO), 1.81-1.88 (1H, m, C*H*CHO), 2.76-2.85 (1H, m, C*H*CH$_2$), 2.90-2.97 (1H, m, C*H*CH$_2$), 3.51 (1H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.53 (1H, dd, *J* = 11.7, 6.0 Hz, CHOBn), 3.80 (3H, s, $OCH_3$), 4.22 (1H, ddd, *J* = 9.4, 6.0, 3.1 Hz, 4-H), 4.33 (1H, q, *J* = 6.0 Hz, 5-H), 4.50 (1H, d, *J* = 12.1 Hz, CHPh), 4.59 (1H, d, *J* = 12.1 Hz, CHPh), 7.28 (2H, d, *J* = 8.6 Hz, Ph 3,5-$H_2$), 7.30-7.36 (5H, m, Ph-$H_5$), 7.52 (2H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 444.1746 (M + H) ($C_{25}H_{29}{}^{37}ClNO_4$ requires 444.1755),

442.1764 (M + H) ($C_{25}H_{29}{}^{35}ClNO_4$ requires 442.1785), 428/426 (M - $CH_3$), 386/384 (M – $C_2H_3NO$), 391 (M – $CH_3Cl$), 91 (Bn). A solution of sodium methoxide (0.40 g, 8 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.80 g, 8 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (83) (2.3 g, 5.2 mmol) for 2 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (101) (0.60 g, 25%); $R_f$ = 0.63 as a buff solid: mp 55-56 °C; NMR $\delta_H$ 1.28 (6H, s, $CMe_2$), 1.62-1.71 (2H, m, $CH_2CHO$), 2.25 (1H, ddd, $J$ = 13.5, 10.1, 5.9 Hz, $CHCH_2$), 2.51 (1H, ddd, $J$ = 13.5, 10.1, 5.9 Hz, $CHCH_2$), 3.43 (2H, d, $J$ = 5.9 Hz, $CH_2OBn$), 4.01 (1H, ddd, $J$ = 10.1, 5.9, 4.3 Hz, 4-H), 4.21 (1H, q, $J$ = 5.9 Hz, 5-H), 4.47 (1H, d, $J$ = 12.1 Hz, CHPh), 4.56 (1H, d, $J$ = 12.1 Hz, CHPh), 4.82 (2H, br, $NH_2$), 5.23 (2H, br, $NH_2$), 7.14 (1H, d, $J$ = 8.2 Hz, Ph 3-H), 7.15 (1H, d, $J$ = 8.2 Hz, Ph 5-H), 7.27-7.37 (5H, m, Ph-$H_5$), 7.39 (2H, d, $J$ = 8.2 Hz, Ph 2,6-$H_2$); MS *m/z* 471.1992 (M + H) ($C_{25}H_{30}{}^{37}ClN_4O_3$ requires 471.1976), 469.2005 (M + H) ($C_{25}H_{30}{}^{35}ClN_4O_4$ requires 469.2006), 236/234 (M – $C_{14}H_{18}O_3$), 91 (Bn).

**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (84) and 2,4-diamino-6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4yl)-ethyl)-5-(4-bromophenyl)pyrimidine (102)**



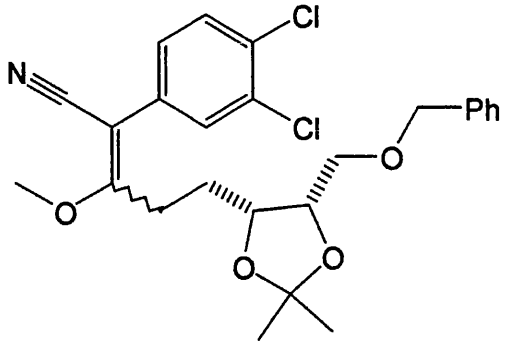

**(84)**



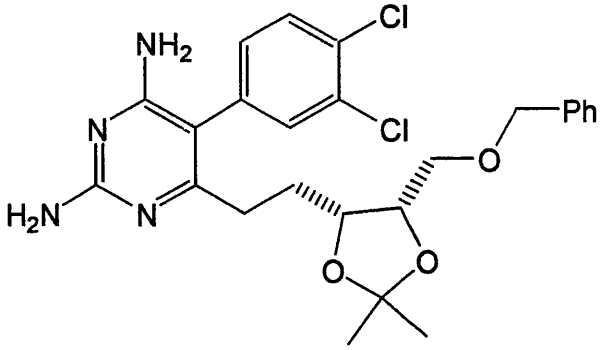

**(102)**

A solution of compound (63) (1.5 g, 3 mmol) in THF (5 ml) was methylated with diazomethane in the same method described above to afford (84) (1.5 g, 99%) as a pale yellow oil; NMR $\delta_H$ 1.36 (3H, s, Me), 1.45 (3H, s, Me), 1.75-1.88 (2H, m, C*H*$_2$CHO), 2.74-2.86 (1H, m, C*H*CH$_2$), 2.89-2.97 (1H, m, C*H*CH$_2$), 3.51 (1H, dd, *J* = 11.7, 5.9 Hz, CHOBn), 3.53 (1H, dd, *J* = 11.7, 5.9 Hz, CHOBn), 3.80 (3H, s, $OCH_3$), 4.21 (1H, ddd, *J* = 9.4, 5.9, 3.1 Hz, 4-H), 4.32 (1H, q, *J* = 5.9 Hz, 5-H), 4.50 (1H, d, *J* = 11.9 Hz, CHPh), 4.59 (1H, d, *J* = 11.9 Hz, CHPh), 7.27-7.32 (5H, m, Ph-H$_5$), 7.43 (2H, d, *J* = 8.6 Hz, Ph 3,5-H$_2$), 7.47 (2H, d, *J* = 9.0 Hz, Ph 2,6-H$_2$); MS *m/z* 488.1255 (M + H) ($C_{25}H_{29}{}^{81}BrNO_4$ requires 488.1259), 486.1263 (M + H) ($C_{25}H_{29}{}^{79}BrNO_4$ requires 486.1279), 430/428 (M - $C_2H_3NO$), 391 (M – $CH_3Br$), 91 (Bn). A solution of sodium methoxide (0.25 g, 4.6 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.44 g, 4.6 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (84) (1.5 g, 3 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9.5:0.5) to afford (102) (0.90 g, 57%); $R_f$ = 0.34 as a buff solid: mp 62-64 °C; IR $\nu_{max}$ 3462 ($NH_2$), 1635 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.26 (6H, s, $CMe_2$), 1.58-1.63 (2H, m, C*H*$_2$CHO), 2.22 (1H, ddd, *J* = 13.3, 10.1, 5.9 Hz, C*H*CH$_2$), 2.49 (1H, ddd, *J* = 13.3, 10.1, 5.9 Hz, C*H*CH$_2$), 3.41 (2H, d, *J* = 5.9 Hz, $CH_2OBn$), 4.00 (1H, ddd, *J* = 9.8, 5.9, 3.5 Hz, 4-H), 4.20 (1H, q, *J* = 5.9 Hz, 5-H), 4.45 (1H, d, *J* = 12.1 Hz, CHPh), 4.54 (1H, d, *J* = 12.1 Hz, CHPh), 4.65 (2H, br, $NH_2$), 5.06 (2H, br, $NH_2$), 7.04 (1H, d, *J* = 7.8 Hz, Ph 3-H), 7.06 (1H, d, *J* = 8.2 Hz, Ph 5-H), 7.23-7.33 (5H, m, Ph-H$_5$), 7.50 (2H, d, *J* = 8.2 Hz, Ph 2,6-H$_2$); MS *m/z* 515.1483 (M + H) ($C_{25}H_{30}{}^{81}BrN_4O_3$ requires 515.1480), 513.1497 (M + H) ($C_{25}H_{30}{}^{79}BrN_4O_3$ requires 513.1501), 499/497 (M – $CH_3$), 280/278 (M – $C_{14}H_{18}O_3$), 91 (Bn).

**(4*R*,5*S*)-5-Benzyloxymethyl-4-(4-(3,4-dichlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (85) and 2,4-diamino-6-(2-((4*R*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-(3,4-dichlorophenyl)pyrimidine (103)**




(85)

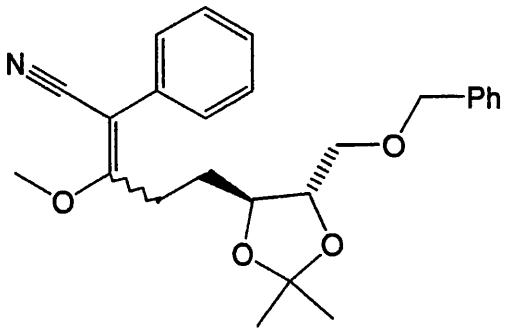



(103)

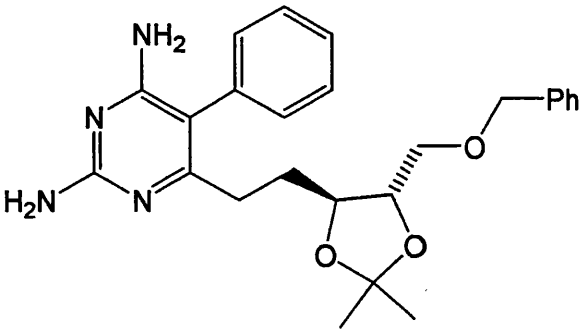
A solution of compound (64) (1.5 g, 3.2 mmol) in THF (5 ml) was methylated with diazomethane in the same method described above to afford (85) (1.5 g, 97%) as a pale yellow oil; NMR $\delta_H$ 1.36 (3H, s, Me), 1.45 (3H, s, Me), 1.73-1.80 (1H, m, C*H*CHO), 1.82-1.88 (1H, m, C*H*CHO), 2.76-2.85 (1H, m, C*H*$CH_2$), 2.90-2.98 (1H, m, C*H*$CH_2$), 3.51 (1H, dd, $J$ = 11.3, 6.0 Hz, CHOBn), 3.53 (1H, dd, $J$ = 11.3, 6.0 Hz, CHOBn), 3.84 (3H, s, $OCH_3$), 4.21 (1H, ddd, $J$ = 9.4, 6.0, 3.1 Hz, 4-H), 4.32 (1H, q, $J$ = 6.0 Hz, 5-H), 4.49 (1H, d, $J$ = 12.1 Hz, CHPh), 4.58 (1H, d, $J$ = 12.1 Hz, CHPh), 7.26-7.32 (5H, m, Ph-$H_5$), 7.37 (1H, d, $J$ = 8.6 Hz, Ph 5-H), 7.42 (1H, dd, $J$ = 8.6, 2.1 Hz, Ph 6-H), 7.73 (1H, d, $J$ = 2.1 Hz, Ph 2-H); MS *m/z* 480.1319 (M + H) ($C_{25}H_{28}{}^{37}Cl_2NO_4$ requires 480.1336), 478.1344 (M + H) ($C_{25}H_{28}{}^{37}Cl\ {}^{35}ClNO_4$ requires 478.1365), 476.1367 (M + H) ($C_{25}H_{28}{}^{35}Cl_2NO_4$ requires 476.1395), 422/420/418 (M - $C_2H_3NO$), 391 (M – $CH_2Cl_2$), 91 (Bn). A solution of sodium methoxide (0.12 g, 2.2 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.21 g, 2.2 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (85) (0.7 g, 1.5 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 19:1) to afford (103) (0.40 g, 47%); $R_f$ = 0.24 as a buff solid: mp 67-69 °C; IR $\nu_{max}$ 3411 ($NH_2$), 1637 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.27 (6H, s, $CMe_2$), 1.54-1.65 (1H, m,

C*H*CHO), 1.68-1.78 (1H, m, C*H*CHO), 2.18-2.32 (1H, m, C*H*$CH_2$), 2.42-2.52 (1H, m, C*H*$CH_2$), 3.44 (2H, d, $J$ = 6.0 Hz, $CH_2OBn$), 3.99 (1H, ddd, $J$ = 9.8, 6.0, 3.5 Hz, 4-H), 4.20 (1H, q, $J$ = 6.0 Hz, 5-H), 4.47 (1H, d, $J$ = 12.3 Hz, CHPh), 4.56 (1H, d, $J$ = 12.3 Hz, CHPh), 4.85 (2H, br, $NH_2$), 5.21 (2H, br, $NH_2$), 7.03 (5H, dd, $J$ = 8.0, 2.0 Hz, Ph 6-H), 7.26-7.32 (5H, m, Ph-$H_5$), 7.35 (1H, d, $J$ = 2.0 Hz, Ph 2-H), 7.46 (1H, d, $J$ = 8.0 Hz, Ph 5-H); MS *m/z* 507.1563 (M + H) ($C_{25}H_{29}{}^{37}Cl_2N_4O_3$ requires 507.1557), 505.1586 (M + H) ($C_{25}H_{29}{}^{37}Cl\ {}^{35}ClN_4O_3$ requires 505.1587), 503.1617 (M + H) ($C_{25}H_{29}{}^{35}Cl_2N_4O_3$ requires 503.1616), 272/270/268 (M – $C_{14}H_{18}O_3$), 91 (Bn).

**(4*S*,5*S*)-5-Benzyloxymethyl-4-(4-cyano-3-methoxy-4-phenylbut-3-enyl)-2,2-dimethyl-1,3-dioxolane (86) and 2,4-diamino-6-(2-((4*S*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine (104)**




**(86)**

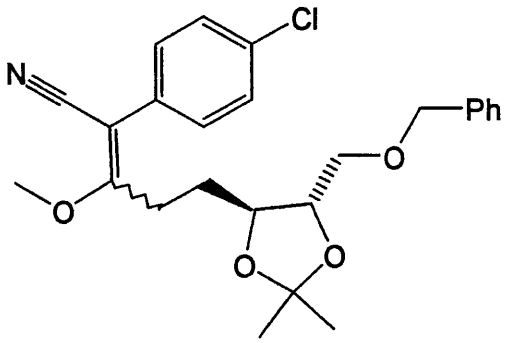



**(104)**

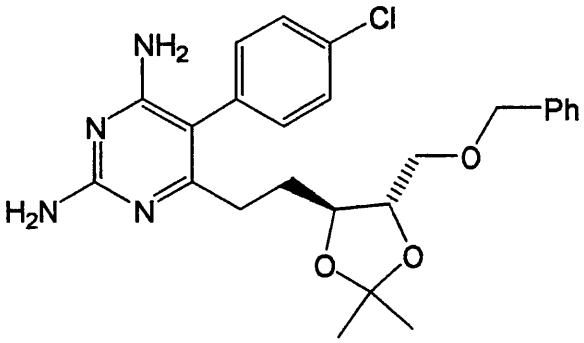
A solution of compound (65) (1.22 g, 3.1 mmol) in diethyl ether (5 ml) was treated with a solution of diazomethane (~0.2 g, 6 mmol) prepared from N-methyl-N-nitroso-p-toluenesulfonamide and KOH (using Minidiazald apparatus) in diethyl ether (20 ml) and the mixture was kept overnight at 10 °C. The excess of diazomethane was destroyed by dropwise addition of acetic acid (30% in THF), and the solvent was evaporated off to afford (86) (1.0 g, 79%) as a pale yellow oil; IR $\nu_{max}$ 2208 (CN), 1605 (C=C) $cm^{-1}$; NMR $\delta_H$ 1.45 (3H, s, Me), 1.49 (3H, s, Me), 1.79-1.87 (1H, m, C*H*CHO), 1.94-2.02 (1H, m, C*H*CHO), 2.40-2.59 (2H, m, C*H*$_2$$CH_2$), 3.55-3.60 (2H, m, 4,5-$H_2$), 3.75 (3H, s, $OCH_3$), 3.81-3.85 (2H, m, $CH_2OBn$), 4.55 (1H, d, $J$ = 12.7 Hz, CHPh), 4.58 (1H, d, $J$ = 12.7 Hz, CHPh), 7.14-7.45 (10H, m, 2 × Ph-$H_5$); MS *m/z* 408.2184 (M + H) ($C_{25}H_{30}NO_4$ requires 408.2174), 391 (M - $CH_4$),

380 (M – CHN), 91 (Bn). A solution of sodium methoxide (0.20 g, 3.7 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.35 g, 3.7 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (86) (1.0 g, 2.5 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (104) (0.70 g, 67%); $R_f$ = 0.5 as a hygroscopic pale yellow solid; IR $\nu_{max}$ 3454 ($NH_2$), 1664 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.26 (3H, s, Me), 1.29 (3H, s, Me), 1.71-1.91 (2H, m, $CH_2CHO$), 2.33 (1H, ddd, $J$ = 13.5, 10.3, 5.8 Hz, $CHCH_2$), 2.45 (1H, ddd, $J$ = 13.5, 10.5, 5.7 Hz, $CHCH_2$), 3.44 (2H, d, $J$ = 4.6 Hz, $CH_2OBn$), 3.67 (1H, dt, $J$ = 7.9, 4.6 Hz, 4-H), 3.75 (1H, q, $J$ = 4.6 Hz, 5-H), 4.28 (1H, d, $J$ = 12.1 Hz, CHPh), 4.54 (1H, d, $J$ = 12.1 Hz, CHPh), 4.64 (2H, br, $NH_2$), 5.14 (2H, br, $NH_2$), 7.19-7.43 (10H, m, 2 × Ph-$H_5$); MS *m/z* 435.2398 (M + H) ($C_{25}H_{31}N_4O_3$ requires 435.2396), 327 (M – $C_7H_7O$), 91 (Bn).

**(4*S*,5*S*)-5-Benzyloxymethyl-4-(4-(4-chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (87) and 2,4-diamino-6-(2-((4*S*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4yl)ethyl)-5-(4-chlorophenyl)pyrimidine (105)**




(87)

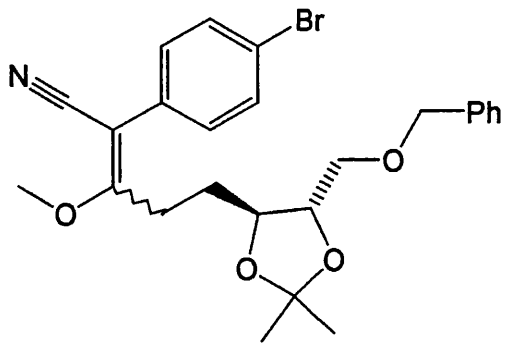



(105)

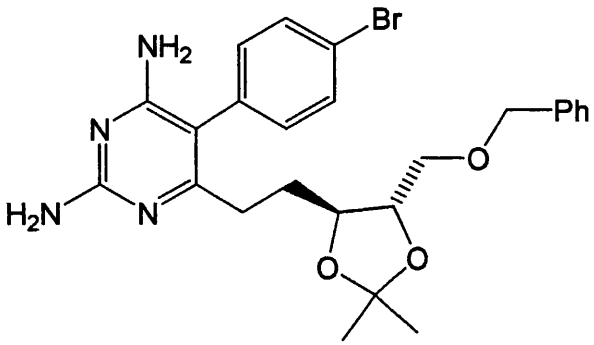
A solution of compound (66) (0.22 g, 0.5 mmol) in THF (5 ml) was methylated with diazomethane by the same method described above to afford (87) (0.20 g, 91%) as a pale yellow oil; NMR $\delta_H$ 1.39 (3H, s, Me), 1.41 (3H, s, Me), 1.62-1.75 (2H, m, $CH_2CHO$), 2.86-2.96 (2H, m, $CH_2CH_2$), 3.52-3.65 (2H, m, 4,5-$H_2$), 3.81 (3H, s,

$OCH_3$), 3.84-3.93 (2H, m, $CH_2OBn$), 4.54 (1H, d, $J$ = 11.1 Hz, CHPh), 4.58 (1H, d, $J$ = 11.1 Hz, CHPh), 7.24-7.38 (9H, m, Ph-$H_5$ + Ph-$H_4$). A solution of sodium methoxide (24 mg, 0.5 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (43 mg, 0.5 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (51) (0.20 g, 0.5 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (105) (0.10 g, 48%); $R_f$ = 0.87 as a hygroscopic pale yellow solid; IR $\nu_{max}$ 3475, 3414 ($NH_2$), 1618 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.28 (3H, s, Me), 1.31 (3H, s, Me), 1.68-1.77 (1H, m, C*H*CHO), 1.80-1.86 (1H, m, C*H*CHO), 2.30 (1H, ddd, $J$ = 13.5, 10.5, 5.7 Hz, C*H*$CH_2$), 2.45 (1H, ddd, $J$ = 13.5, 10.5, 5.7 Hz, C*H*$CH_2$), 3.41-3.49 (2H, m, $CH_2OBn$), 3.3.66 (1H, dt, $J$ = 8.2, 3.5 Hz, 4-H), 3.51 (1H, m, 5-H), 4.50 (1H, d, $J$ = 12.1 Hz, CHPh), 4.54 (1H, d, $J$ = 12.1 Hz, CHPh), 4.69 (2H, br, $NH_2$), 5.11 (2H, br, $NH_2$), 7.10-7.35 (9H, m, Ph-$H_5$ + Ph-$H_4$); MS *m/z* 471.1979 (M + H) ($C_{25}H_{30}{}^{37}ClN_4O_3$ requires 471.1976), 469.1999 (M + H) ($C_{25}H_{30}{}^{35}ClN_4O_3$ requires 469.2006), 363/361 (M – $C_7H_7O$), 91 (Bn).

**(4*S*,5*S*)-5-Benzyloxymethyl-4-(4-(4-bromophenyl)-4-cyano-3-methoxybut-3-enyl)-2,2-dimethyl-1,3-dioxolane (88) and 2,4-diamino-6-(2-((4*S*,5*S*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4yl)-ethyl)-5-(4-bromophenyl)pyrimidine (106)**



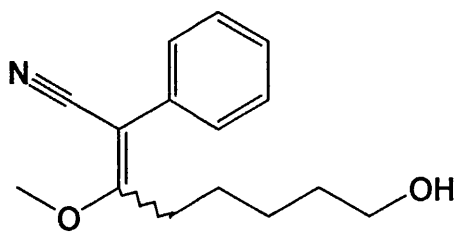

**(88)**



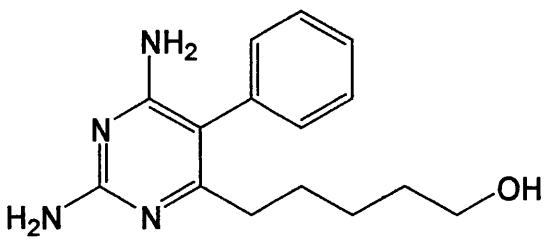

**(106)**

A solution of compound (67) (1.7 g, 3.6 mmol) in diethyl ether (5 ml) was methylated with diazomethane in the same method described above to afford (88) (1.6 g, 93%) as a pale yellow oil; NMR $\delta_H$ 1.39 (3H, s, Me), 1.41 (3H, s, Me), 1.78-1.86 (2H, m, C*H*$_2$CHO), 2.85-2.95 (2H, m, C*H*$_2$CH$_2$), 3.52-3.68 (2H, m, 4,5-H$_2$), 3.81 (3H, s, OCH$_3$), 3.84-3.94 (2H, m, CH$_2$OBn), 4.54 (1H, d, $J$ = 12.1 Hz, CHPh), 4.59 (1H, d, $J$ = 12.1 Hz, CHPh), 7.22-7.42 (9H, m, Ph-H$_5$ + Ph-H$_4$); MS *m/z* 488.1255 (M + H) ($C_{25}H_{29}{}^{81}BrNO_4$ requires 488.1259), 486.1258 (M + H) ($C_{25}H_{29}{}^{79}BrNO_4$ requires 486.1279), 391 (M – $CH_3Br$), 91 (Bn). A solution of sodium methoxide (0.27 g, 5 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.50 g, 5 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (88) (1.6 g, 3.3 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (106) (0.90 g, 53%); $R_f$ = 0.52 as a hygroscopic buff solid; NMR $\delta_H$ 1.31 (3H, s, Me), 1.34 (3H, s, Me), 1.71-1.80 (1H, m, C*H*CHO), 1.83-1.92 (1H, m, C*H*CHO), 2.33 (1H, ddd, $J$ = 13.7, 10.5, 5.9 Hz, C*H*CH$_2$), 2.48 (1H, ddd, $J$ = 13.7, 10.5, 5.9 Hz, C*H*CH$_2$), 3.45-3.53 (2H, m, CH$_2$OBn), 3.70 (1H, dt, $J$ = 7.8, 3.5 Hz, 4-H), 3.75-3.80 (1H, m, 5-H), 4.51 (1H, d, $J$ = 12.1 Hz, CHPh), 4.57 (1H, d, $J$ = 12.1 Hz, CHPh), 4.76 (2H, br, NH$_2$), 5.18 (2H, br, NH$_2$), 7.09 (2H, d, $J$ = 8.6 Hz, Ph 3,5-H$_2$), 7.28-7.38 (5H, m, Ph-H$_5$), 7.54 (2H, d, $J$ = 8.6 Hz, Ph 2,6-H$_2$); MS *m/z* 515.1488 (M + H) ($C_{25}H_{30}{}^{81}BrN_4O_3$ requires 515.1480), 513.1500 (M + H) ($C_{25}H_{30}{}^{79}BrN_4O_4$ requires 513.1501), 487/485 (M – $C_2H_5$), 407/405 (M - $C_7H_7O$), 91 (Bn).

**1-Cyano-7-hydroxy-2-methoxy-1-phenylhept-1-ene (89) and 2,4-diamino-6-(5-hydroxypentyl)-5-phenylpyrimidine (107)**



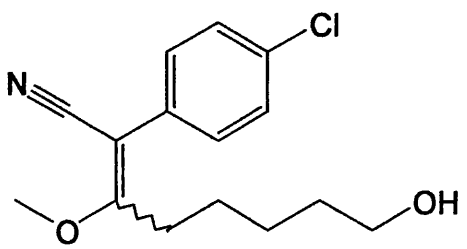

**(89)**



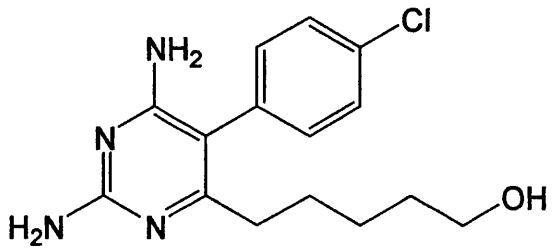

**(107)**

A solution of compound (68) (0.70 g, 3 mmol) was methylated with diazomethane in the same method described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (89) (0.60 g, 78%); $R_f$ = 0.58 as a pale yellow oil; IR $\nu_{max}$ 3439 (OH), 2204 (CN); MS *m/z* 246.1492 (M + H) ($C_{15}H_{20}NO_2$ requires 246.1494), 228 (M – CHN), 214 (M – $CH_3O$), 185 (M – $C_2H_6NO$). A solution of sodium methoxide (0.10 g, 2 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.20 g, 2 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (89) (0.50 g, 2 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/ methanol, 8:2) to afford (107) (0.20 g, 36%); $R_f$ = 0.50 as a white solid: mp 214-216 °C; IR $\nu_{max}$ 3420, 3331 ($NH_2$), 3177 (OH), 1619 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.27 (2H, qn, *J* = 7.2 Hz, 3-$H_2$), 1.45 (2H, m, 4-$H_2$), 1.55 (2H, qn, *J* = 7.2 Hz, 2-$H_2$), 2.28 (2H, t, *J* = 7.2 Hz, 1-$H_2$), 3.56 (2H, t, *J* = 6.4 Hz, 5-$H_2$), 4.59 (2H, br, $NH_2$), 4.98 (2H, br, $NH_2$), 7.21 (2H, d, *J* = 7.2 Hz, Ph 3,5-$H_2$), 7.37 (1H, t, *J* = 7.2 Hz, Ph 4-H), 7.44 (2H, t, *J* = 7.2 Hz, Ph 2,6-$H_2$); NMR ($CD_3OD$) $\delta_C$ 25.38 ($CH_2$), 28.49 ($CH_2$), 31.81 ($CH_2$), 33.79 ($CH_2$), 61.33 (5-$CH_2$), 108.29 (Pyr 5-C), 127.67 (Ph CH), 128.95 (2 × Ph CH), 130.51 (2 × Ph CH), 134.76 (Ph 1-C), 161.32 (Pyr 2-C), 162.98 (Pyr 4-C), 165.09 (Pyr 6-C); MS *m/z* 273.1704 (M + H) ($C_{15}H_{20}N_4O$ requires 273.1715), 213 (M – $C_3H_7O$), 200 (M – $C_4H_8O$).

**1-(4-Chlorophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (90) and 2,4-diamino-5-(4-chlorophenyl)-6-(5-hydroxypentyl)pyrimidine (108)**




(90)

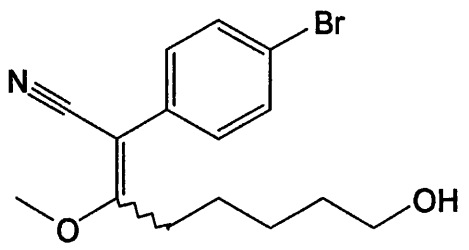



(108)

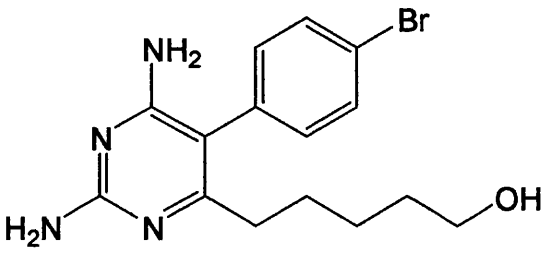
A solution of compound (69) (0.20 g, 0.8 mmol) was methylated with diazomethane in the same method described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (90) (0.13 g, 62%); $R_f$ = 0.29

as a pale yellow oil; NMR $\delta_H$ 1.52-1.80 (6H, m, 4,5,6-$H_6$), 2.77 (2H, t, $J$ = 7.8 Hz, 3-$H_2$), 3.68 (2H, t, $J$ = 6.2 Hz, 7-$H_2$), 3.85 (3H, s, $OCH_3$), 7.29 (2H, d, $J$ = 8.6 Hz, Ph 3,5-$H_2$), 7.54 (2H, d, $J$ = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 282.1077 (M + H) ($C_{15}H_{19}{}^{37}ClNO_2$ requires 280.1074), 280.1102 (M + H) ($C_{15}H_{19}{}^{35}ClNO_2$ requires 280.1104), 264/262 (M – $CH_3$), 221/219 (M – $C_2H_6NO$). A solution of sodium methoxide (27 mg, 0.5 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (47 mg, 0.5 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (90) (0.14 g, 0.5 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (108) (90 mg, 59%); $R_f$ = 0.37 as a white solid: mp 165-166 °C; IR $\nu_{max}$ 3407, 3329 ($NH_2$), 3174 (OH), 1631 (C=N) $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.11 (2H, qn, $J$ = 7.4 Hz, 3-$H_2$), 1.26 (2H, qn, $J$ = 7.4 Hz, 4-$H_2$), 1.42 (2H, qn, $J$ = 7.4 Hz, 2-$H_2$), 2.07 (2H, t, $J$ = 7.4 Hz, 1-$H_2$), 3.29 (2H, t, $J$ = 7.4 Hz, 5-$H_2$), 4.29 (1H, br, OH), 5.64 (2H, br, $NH_2$), 5.94 (2H, br, $NH_2$) 7.18 (2H, d, $J$ = 8.2 Hz, Ph 3,5-$H_2$), 7.47 (2H, d, $J$ = 8.2 Hz, Ph 2,6-$H_2$); NMR ($(CD_3)_2SO$) $\delta_C$ 25.88 (3-$CH_2$), 28.58 (2-$CH_2$), 32.69 (4-$CH_2$), 34.63 (1-$CH_2$), 61.00 (5-$CH_2$), 106.16 (Pyr 5-C), 129.34 (Ph 3,5-C), 132.25 (Ph 2,6-C), 134.76 (Ph 1-C), 135.47 (Ph 4-C), 162.44 (Pyr 2-C), 162.47 (Pyr 4-C), 165.87 (Pyr 6-C); MS *m/z* 309.1310 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_2$ requires 309.1296), 307.1335 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_2$ requires 307.1325), 236/234 (M - $C_4H_8O$).

**1-(4-Bromophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (91) and 2,4-diamino-5-(4-bromophenyl)-6-(5-hydroxypentyl)pyrimidine (109)**




**(91)**

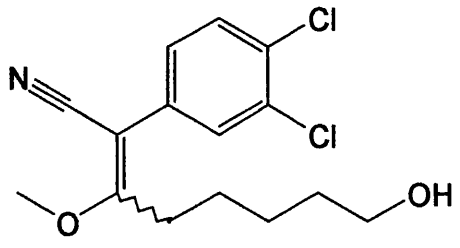



**(109)**

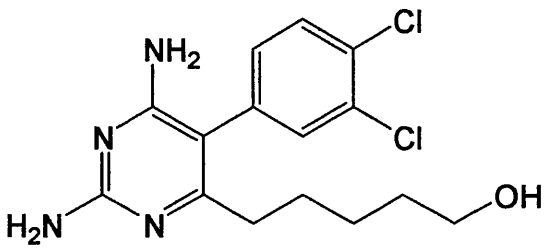
A solution of compound (70) (0.30 g, 1 mmol) was methylated with diazomethane in the same method described before. The resulting yellow oil was purified by

chromatography (ethyl acetate/hexane, 3:1) to afford (91) (0.10 g, 32%); $R_f$ = 0.29 as a pale yellow oil; NMR $\delta_H$ 1.53-1.75 (6H, m, 4,5,6-$H_6$), 2.76 (2H, t, $J$ = 7.0 Hz, 3-$H_2$), 3.69 (2H, t, $J$ = 7.0 Hz, 7-$H_2$), 3.85 (3H, s, $OCH_3$), 7.44 (2H, d, $J$ = 8.8 Hz, Ph 3,5-$H_2$), 7.48 (2H, d, $J$ = 8.8 Hz, Ph 2,6-$H_2$); MS *m/z* 326.0583 (M + H) ($C_{15}H_{19}{}^{81}BrNO_2$ requires 326.0578), 324.0596 (M + H) ($C_{15}H_{19}{}^{79}BrNO_2$ requires 324.0599), 293 (M - $CH_2O$), 259 (M - $CH_3Br$). A solution of sodium methoxide (32 mg, 0.6 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (57 mg, 0.6 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (91) (0.2 g, 0.6 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (109) (90 mg, 43%); $R_f$ = 0.52 as a white solid: mp 177-178 °C; IR $\nu_{max}$ 3550 (OH), 3468, 3414 ($NH_2$), 1617 (C=N) $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.11 (2H, qn, $J$ = 7.4 Hz, 3-$H_2$), 1.25 (2H, m, 4-$H_2$), 1.42 (2H, qn, $J$ = 7.4 Hz, 2-$H_2$), 2.07 (2H, t, $J$ = 7.4 Hz, 1-$H_2$), 3.27 (2H, t, $J$ = 6.4 Hz, 5-$H_2$), 4.30 (1H, br, OH), 5.74 (2H, br, $NH_2$), 6.00 (2H, br, $NH_2$), 7.11 (2H, d, $J$ = 8.4 Hz, Ph 2,6-$H_2$), 7.57 (2H, d, $J$ = 8.4 Hz, Ph 3,5-$H_2$); NMR (TFA salt) ($(CD_3)_2SO$) $\delta_C$ 25.33 ($CH_2$), 27.72 ($CH_2$), 30.33 ($CH_2$), 32.21 ($CH_2$), 60.68 (5-C), 108.00 (Pyr 5-C), 115.78 ($CF_3$, q, $J$ = 289 Hz) 122.75 (Ph 4-C), 130.74 (Ph 1-C), 132.74 (2 × Ph CH), 133.24 (2 × Ph CH), 153.43 (Pyr 2-C), 155.31 (Pyr 4-C), 158 ($CF_3CO$, q, $J$ = 38 Hz), 164.30 (Pyr 6-C); MS *m/z* 353.0807 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O$ requires 353.0800), 351.0816 (M + H) ($C_{15}H_{20}{}^{79}BrN_4O$ requires 351.0820).

**1-(3,4-Dichlorophenyl)-1-cyano-7-hydroxy-2-methoxyhept-1-ene (92) and 2,4-diamino-5-(3,4-dichlorophenyl)-6-(5-hydroxypentyl)pyrimidine (110)**



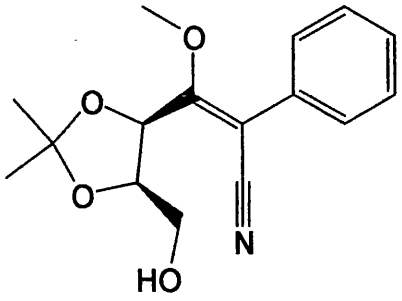

**(92)**



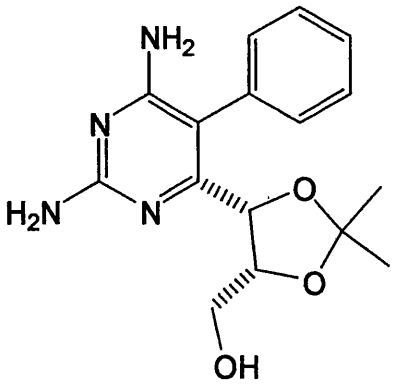

**(110)**

A solution of compound (71) (3.0 g, 10 mmol) was methylated with diazomethane in the same method described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (92) (2.2 g, 68%); $R_f$ = 0.42 as a pale yellow oil; NMR $\delta_H$ 1.41-1.50 (4H, m, 5,6-$H_4$), 1.62 (2H, qn, $J$ = 7.6 Hz, 4-$H_2$), 2.75 (2H, t, $J$ = 7.6 Hz, 3-$H_2$), 3.41 (2H, t, $J$ = 6.0 Hz, 7-$H_2$), 3.95 (3H, s, $OCH_3$), 7.50 (1H, dd, $J$ = 8.6, 2.0 Hz, Ph 6-H), 7.63 (1H, d, $J$ = 8.6 Hz, Ph 5-H), 7.75 (1H, d, $J$ = 2.0 Hz, Ph 2-H); MS *m/z* 318.0686 (M + H) ($C_{15}H_{18}{}^{37}Cl_2NO_2$ requires 318.0655), 316.0689 (M + H) ($C_{15}H_{18}{}^{37}Cl^{35}ClNO_2$ requires 316.0685), 314.0715 (M + H) ($C_{15}H_{18}{}^{35}Cl_2NO_2$ requires 314.0714), 291/289/287 (M – CN), 229 (M - $CH_2Cl_2$). A solution of sodium methoxide (0.30 g, 6 mmol) in 2-methoxyethanol (10 ml) was added to a solution of guanidine hydrochloride (0.60 g, 6 mmol) in 2-methoxyethanol (10 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (92) (2.0 g, 6 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (110) (0.90 g, 43%); $R_f$ = 0.84 as a white solid: mp 94-95 °C; IR $\nu_{max}$ 3499 (OH), 3419, 3333 ($NH_2$), 1622 (C=N) $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.12 (2H, qn, $J$ = 7.4 Hz, 3-$H_2$), 1.25 (2H, m, 4-$H_2$), 1.42 (2H, qn, $J$ = 7.4 Hz, 2-$H_2$), 2.07 (2H, t, $J$ = 7.4 Hz, 1-$H_2$), 3.27 (2H, q, $J$ = 5.6 Hz, 5-$H_2$), 4.28 (1H, t, $J$ = 5.6 Hz, OH), 5.72 (2H, br, $NH_2$), 5.90 (2H, br, $NH_2$), 7.11 (1H, dd, $J$ = 8.2, 2.2 Hz, Ph 6-H), 7.36 (1H, d, $J$ = 2.2 Hz, Ph 2-H), 7.62 (1H, d, $J$ = 8.2 Hz, Ph 5-H); NMR ($(CD_3)_2SO$) $\delta_C$ 25.87 ($CH_2$), 28.50 ($CH_2$), 32.70 ($CH_2$), 34.64 ($CH_2$), 61.02 (5-$CH_2$), 105.23 (Pyr 5-C), 130.21 (Ph C), 131.37 (Ph CH), 131.74 (Ph C), 131.81 (Ph CH), 133.19 (Ph CH), 137.66 (Ph C), 162.36 (Pyr 2-C), 162.69 (Pyr 4-C), 165.96 (Pyr 6-C); MS *m/z* 345.0885 (M + H) ($C_{15}H_{19}{}^{37}Cl_2N_4O$ requires 345.0876), 343.0901 (M + H) ($C_{15}H_{19}{}^{37}Cl^{35}ClN_4O$ requires 343.0906), 341.0927 (M + H) ($C_{15}H_{19}{}^{35}Cl_2N_4O$ requires 341.0935), 285/283/281 (M - $C_3H_7O$), 272/270/268 (M – $C_4H_8O$).

**(4*R*,5*R*)-4-(2-Cyano-1-methoxy-2-phenylethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (93) and 2,4-diamino-6-((4*S*,5*R*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)-5-phenylpyrimidine (111)**




(93)

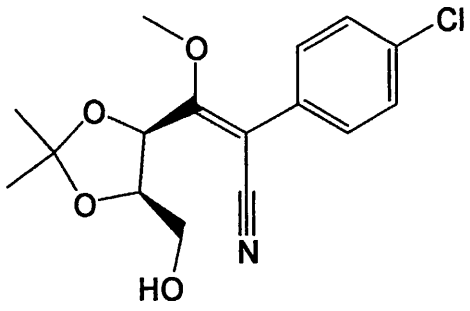



(111)

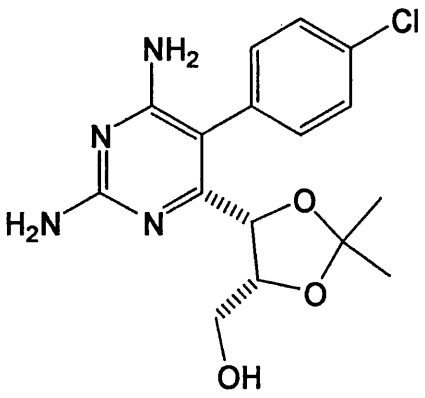
A solution of compound (72) (0.25 g, 0.9 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described before to afford (93) (0.22 g, 85%) as a pale yellow oil; IR $\nu_{max}$ 3492 (OH), 2209 (CN) $cm^{-1}$; NMR $\delta_H$ 1.44 (3H, s, Me), 1.58 (3H, s, Me), 3.53 (3H, s, $OCH_3$), 4.39 (1H, dd, $J$ = 11.0, 3.7 Hz, C*H*OH), 4.45 (1H, d, $J$ = 11.0 Hz, C*H*OH), 4.57-4.62 (1H, m, 5-H), 5.37 (1H, d, $J$ = 7.4 Hz, 4-H), 7.30-7.41 (5H, m, Ph-$H_5$); MS *m/z* 290.1388 (M + H) ($C_{16}H_{20}NO_4$ requires 290.1392), 274 (M – $CH_3$), 258 (M - $CH_3O$). A solution of sodium methoxide (50 mg, 0.9 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (80 mg, 0.9 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (93) (0.26 g, 0.9 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (111) (0.10 g, 48%); $R_f$ = 0.39 as a pale yellow solid: mp 214-216 °C; IR $\nu_{max}$ 3492, 3465, 3422, 3318 ($NH_2$), 3178 (OH), 1624 (C=N) $cm^{-1}$; $[\alpha]^{20}_D$ = +3.3° (c 4, $CHCl_3$); NMR $\delta_H$ 1.21 (3H, s, Me), 1.62 (3H, s, Me), 3.48 (1H, dd, $J$ = 12.7, 2.1 Hz, C*H*OH), 3.57 (1H, dd, $J$ = 12.7, 3.3 Hz, C*H*OH), 3.97 (1H, m, 5-H), 4.79 (1H, d, $J$ = 6.6 Hz, 4-H), 4.90 (2H, br, $NH_2$), 5.16 (2H, br, $NH_2$), 7.10 (1H, d, $J$ = 7.4 Hz, Ph 6-H), 7.31 (1H, d, $J$ = 7.4 Hz, Ph 2-H), 7.41 (1H, t, $J$ = 7.4 Hz, Ph 4-H), 7.47 (2H, t, $J$ = 7.4 Hz, Ph 3,5-$H_2$); MS *m/z*

317.1622 (M + H) ($C_{16}H_{20}N_4O_3$ requires 317.1613), 259 (M – $C_3H_5O$), 215 (M – $C_4H_5O_3$).

**(4*R*,5*R*)-4-(2-(4-Chlorophenyl)-2-cyano-1-methoxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (94) and 2,4-diamino-5-(4-chlorophenyl)-6-((4*S*,5*R*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)pyrimidine (112)**




**(94)**

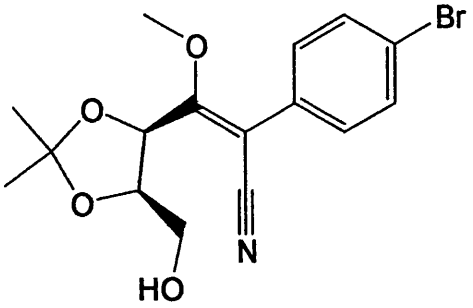



**(112)**

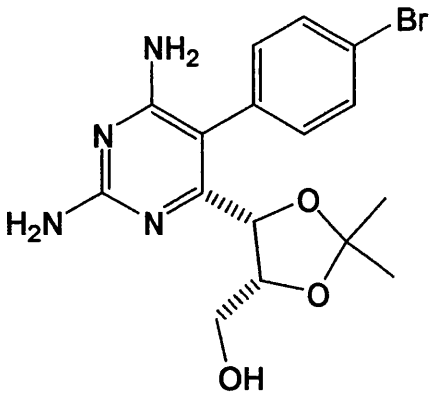
A solution of compound (73) (0.30 g, 0.9 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described before to afford (94) (0.20 g, 69%) as a pale yellow oil; MS *m/z* 326.0989 (M + H) ($C_{16}H_{19}{}^{37}ClNO_4$ requires 326.0973), 324.1014 (M + H) ($C_{16}H_{19}{}^{35}ClNO_4$ requires 324.1002), 307/305 (M - $H_2O$), 265/263 (M – $C_3H_8O$), 238/236 (M – $C_4H_9NO$). A solution of sodium methoxide (46 mg, 0.86 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (82 mg, 0.86 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (94) (0.28 g, 0.86 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (112) (0.15 g, 50%); $R_f$ = 0.92 as a buff solid: mp 172-174 °C; IR $\nu_{max}$ 3497, 3459, 3433, 3396 ($NH_2$), 3217 (OH), 1613 (C=N) $cm^{-1}$; NMR $\delta_H$ 1.24 (3H, s, Me), 1.63 (3H, s, Me), 1.66 (1H, br, OH), 3.47 (1H, dd, *J* = 12.8, 2.3 Hz, C*H*OH), 3.58 (1H, dd, *J* = 12.8, 3.3 Hz, C*H*OH), 3.98 (1H, m, 5-H), 4.66 (2H, br, $NH_2$), 4.77 (1H, d, *J* = 6.2 Hz, 4-H), 4.93 (2H, br, $NH_2$), 7.05 (1H, dd, *J* = 8.8, 2.0 Hz, Ph 3-H), 7.27 (1H, dd, *J* = 9.4, 2.0 Hz, Ph 5-H), 7.43 (1H, dd, *J* = 8.8, 2.0 Hz, Ph 2-H), 7.47 (1H, dd, *J* = 9.4, 2.0 Hz, Ph 6-H); MS *m/z*

353.1218 (M + H) ($C_{16}H_{20}{}^{37}ClN_4O_3$ requires 353.1194), 351.1236 (M + H) ($C_{16}H_{20}{}^{35}ClN_4O_3$ requires 351.1223), 395/293 (M – $C_3H_5O$), 242 (M – $C_3H_5O_2Cl$).

**(4*R*,5*R*)-4-(2-(4-Bromophenyl)-2-cyano-1-methoxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (95) and 2,4-diamino-5-(4-bromophenyl)-6-((4*S*,5*R*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)pyrimidine (113)**




(95)

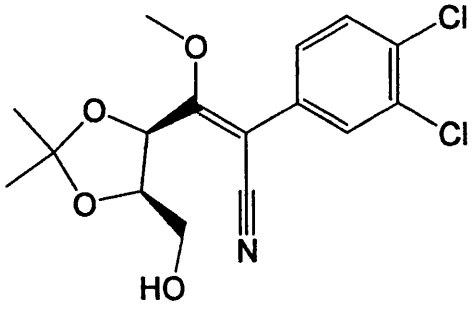



(113)

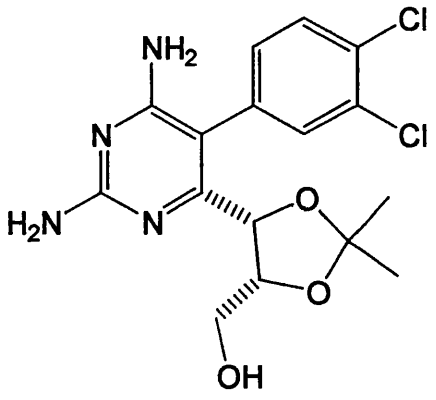
A solution of compound (74) (1.40 g, 3.9 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described before to afford (95) (1.30 g, 91%) as a pale yellow oil; NMR $\delta_H$ 1.44 (3H, s, Me), 1.48 (3H, s, Me), 3.57 (3H, s, $OCH_3$), 4.40 (1H, dd, $J$ = 10.9, 3.5 Hz, C*H*OH), 4.46 (1H, d, $J$ = 10.9 Hz, C*H*OH), 4.55-4.67 (1H, m, 5-H), 5.34 (1H, d, $J$ = 7.0 Hz, 4-H), 7.36 (2H, d, $J$ = 8.6 Hz, Ph 3,5-$H_2$), 7.50 (2H, d, $J$ = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 370.0481 (M + H) ($C_{16}H_{19}{}^{81}BrNO_4$ requires 370.0476), 368.0501 (M + H) ($C_{16}H_{19}{}^{79}BrNO_4$ requires 368.0497), 336 (M – $CH_3O$), 310 (M – $C_2H_3NO$). A solution of sodium methoxide (0.30 g, 5.3 mmol) in 2-methoxyethanol (10 ml) was added to a solution of guanidine hydrochloride (0.51 g, 5.3 mmol) in 2-methoxyethanol (10 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (95) (1.30 g, 3.5 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 8:2) to afford (113) (0.41 g, 29%); $R_f$ = 0.68 as a pale yellow solid: mp 181-183 °C; NMR ($(CD_3)_2O$) $\delta_H$ 1.14 (3H, s, Me), 1.48 (3H, s, Me), 3.45 (2H, d, $J$ = 3.5 Hz, C$H_2$OH), 4.03 (1H, dt, $J$ = 6.4, 3.5 Hz, 5-H), 4.73 (1H, d, $J$ = 6.4 Hz, 4-H), 5.70 (2H, br, $NH_2$), 5.85 (2H, br, $NH_2$), 7.20 (1H, dd, $J$ = 8.1, 2.0 Hz, Ph 3-H), 7.25 (1H, dd, $J$ = 7.7, 2.0 Hz, Ph 5-H),

7.61 (1H, dd, $J$ = 7.7, 2.0 Hz, Ph 6-H), 7.63 (1H, dd, $J$ = 8.1, 2.0 Hz, Ph 2-H); NMR $(CD_3)_2O$) $\delta_C$ 25.44 (Me), 26.37 (Me), 62.49 ($CH_2OH$), 76.46 (CH), 79.81 (CH), 108.35 ($CMe_2$), 108.72 (Pyr 5-C), 121.70 (Ph 4-C), 132.30 (Ph CH), 132.35 (Ph CH), 132.59 (Ph CH), 133.38 (Ph CH), 134.30 (Ph 1-C), 159.21 (Pyr 2-C), 162.29 (Pyr 4-C), 163.21 (Pyr 6-C); MS *m/z* 397.0694 (M + H) ($C_{16}H_{20}{}^{81}BrN_4O_3$ requires 397.0698), 395.0712 (M + H) ($C_{16}H_{20}{}^{79}BrN_4O_3$ requires 395.0718), 339/337 (M – $C_3H_5O$).

**(4*R*,5*R*)-4-(2-(3,4-Dichlorophenyl)-2-cyano-1-methoxyethenyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (96) and 2,4-diamino-5-(3,4-dichlorophenyl)-6-((4*S*,5*R*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)pyrimidine (114)**




(96)

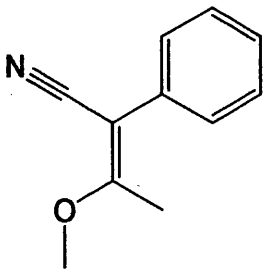



(114)

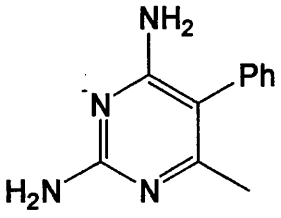
A solution of compound (75) (1.50 g, 4.3 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described before to afford (96) (1.20 g, 78%) as a pale yellow oil; IR $\nu_{max}$ 3534 (OH), 2247 (CN), 1595 (C=C) $cm^{-1}$; NMR $\delta_H$ 1.44 (3H, s, Me), 1.47 (3H, s, Me), 3.72 (3H, s, $OCH_3$), 4.54 (1H, dd, $J$ = 10.7, 4.1 Hz, C*H*OH), 4.73 (1H, d, $J$ = 10.7 Hz, C*H*OH), 4.96 (1H, m, 5-H), 5.52 (1H, d, $J$ = 5.9 Hz, 4-H), 7.15 (1H, d, $J$ = 8.5 Hz, Ph 5-H), 7.37 (1H, dd, $J$ = 8.5, 1.4 Hz, Ph 6-H), 7.40 (1H, d, $J$ = 1.4 Hz, Ph 2-H); MS *m/z* 360.0378 (M - H) ($C_{16}H_{16}{}^{37}Cl_2NO_4$ requires 360.0397), 358.0433 (M - H) ($C_{16}H_{16}{}^{37}Cl^{35}ClNO_4$ requires 358.0426), 356.0452 (M - H) ($C_{16}H_{16}{}^{35}Cl_2NO_4$ requires 356.0456), 346/344/342 (M – $CH_3$), 330/328/326 (M – $CH_3O$). A solution of sodium methoxide (0.15 g, 2.8 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.27 g,

2.8 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (96) (1.0 g, 2.8 mmol) overnight. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (114) (0.50 g, 47%); $R_f$ = 0.42 as a pale yellow solid: mp 181-183 °C; NMR ($CD_3CN$) $\delta_H$ 1.20 (3H, s, Me), 1.49 (3H, s, Me), 3.40-3.42 (2H, m, $CH_2OH$), 4.00-4.05 (1H, m, 5-H), 4.72 (1H, d, $J$ = 7.0 Hz, 4-H), 5.21 (2H, br, $NH_2$), 5.31 (2H, br, $NH_2$), 7.11 (0.5H, dd, $J$ = 8.2, 2.0 Hz, Ph 6-H), 7.21 (0.5H, dd, $J$ = 8.2, 2.0 Hz, Ph 6-H), 7.38 (0.5H, d, $J$ = 2.0 Hz, Ph 2-H), 7.48 (0.5H, d, $J$ = 2.0 Hz, Ph 2-H), 7.60 (0.5H, d, $J$ = 8.2 Hz, Ph 5-H), 7.62 (0.5H, d, $J$ = 8.2 Hz, Ph 5-H); NMR ($(CD_3)_2CO$) $\delta_C$ 24.90 (Me), 25.88 (Me), 61.93 ($CH_2OH$), 76.13 (CH), 79.16 (CH), 107.43 ($CMe_2$), 108.75 (Pyr 5-C), 130.36 (Ph C), 131.07 (Ph CH), 131.18 (Ph C), 131.51 (Ph CH), 132.89 (Ph CH), 135.14 (Ph C), 159.37 (Pyr 2-C), 161.93 (Pyr 4-C), 162.93 (Pyr 6-C); MS *m/z* 389.0778 (M + H) ($C_{16}H_{19}{}^{37}Cl_2N_4O_3$ requires 389.0775), 387.0810 (M + H) ($C_{16}H_{19}{}^{37}Cl^{35}ClN_4O_3$ requires 387.0833), 385.0833 (M + H) ($C_{16}H_{19}{}^{35}Cl_2N_4O_3$ requires 385.0834), 331/329/327 (M –$C_3H_5O$).

**1-Cyano-2-methoxy-1-phenylprop-1-ene (97) and 2,4-diamino-6-methl-5-phenylpyrimidine (115)**




(97)

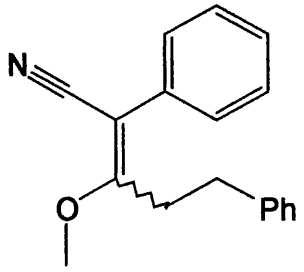



(115)

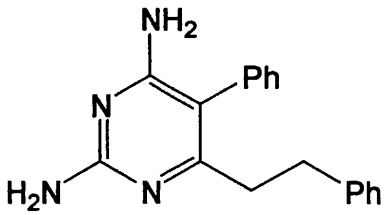
Compound (76) (0.30 g, 2 mmol) in THF (5 ml) was methylated with diazomethane as described before. The resulting yellow oil was purified by chromatography (ethyl acetate/hexane, 2:1) to afford (97) (0.30 g, 87%) as a pale yellow oil; IR $\nu_{max}$ 2204 (CN), 1606 (C=C) $cm^{-1}$; NMR $\delta_H$ 2.45 (3H, s, $CH_3$), 3.85 (3H, s, $OCH_3$), 7.26 (1H, t, $J$ = 7.0 Hz, Ph 4-H), 7.30 (2H, t, $J$ = 7.0 Hz, Ph 3,5-$H_2$), 7.61 (2H, t, $J$ = 7.0 Hz, Ph 2,6-$H_2$); MS *m/z* 174.0921 (M + H) ($C_{11}H_{12}NO$ requires 174.0918), 115 (M –

$C_2H_4NO$). A solution of sodium methoxide (0.10 g, 1.7 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (0.16 g, 1.7 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (97) (0.30 g, 1.7 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:2) to afford (115) (0.13 g, 38%); $R_f$ = 0.42 as a pale yellow solid: mp 250-251 °C (lit.[132] mp 250-251 °C); IR$\nu_{max}$ 3395, 3323 ($NH_2$) $cm^{-1}$; NMR ($(CD_3)_2SO$) $\delta_H$ 1.85 (3H, s, $CH_3$), 5.62 (2H, br, $NH_2$), 6.00 (2H, br, $NH_2$), 7.20 (2H, d, $J$ = 7.3 Hz, Ph 2,6-$H_2$), 7.33 (1H, t, $J$ = 7.3 Hz, Ph 4-H), 7.43 (2H, t, $J$ = 7.3 Hz, Ph 3,5-$H_2$); MS *m/z* 201.1145 (M + H) ($C_{11}H_{13}N_4$ requires 201.1140), 123 (M – $C_6H_5$), 109 (M – $C_7H_7$).

**1-Cyano-2-methoxy-1,4-diphenylbut-1-ene (98) and 2,4-diamino-5-phenyl-6-(2-phenylethyl)pyrimidine (116)**




(98)

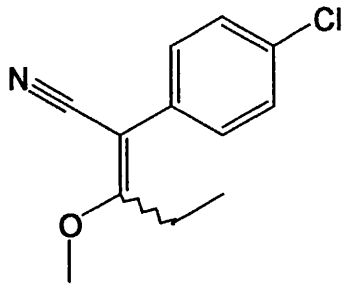



(116)

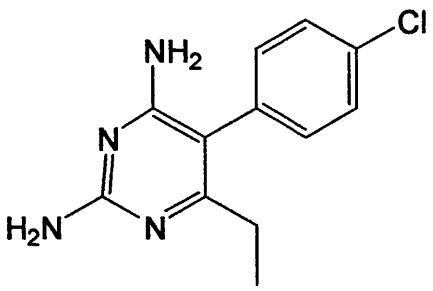
Compound (77) (0.50 g, 2 mmol) in THF (5 ml) was methylated with diazomethane as described before to afford (98) (0.50 g, 95%) as a pale yellow oil; IR $\nu_{max}$ 2204 (CN) $cm^{-1}$; MS *m/z* 264.1390 (M + H) ($C_{18}H_{18}NO$ requires 264.1388), 236 (M – CHN), 204 (M – $C_2H_5NO$), 91 (Bn). A solution of sodium methoxide (80 mg, 1.5 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (140 mg, 1.5 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (98) (0.40 g, 1.5 mmol) overnight. The cooled concentrated solution was purified by chromatography (dichloromethane/methanol, 8:1) to afford (116) (0.14 g, 32%), $R_f$ = 0.42 as a pale

yellow solid mp 116-118 °C; NMR $\delta_H$ 2.54 (2H, t, $J$ = 8.0 Hz, $CH_2$), 2.83 (2H, t, $J$ = 8.0 Hz, $CH_2$), 4.62 (2H, br, $NH_2$), 4.99 (2H, br, $NH_2$), 6.94 (2H, d, $J$ = 6.8 Hz, Ph 2,6-$H_2$), 7.05 (2H, d, $J$ = 6.5 Hz, Ph 2,6-$H_2$), 7.14 (1H, t, $J$ = 6.8 Hz, Ph 4-H), 7.17 (2H, t, $J$ = 6.8 Hz, Ph 3,5-$H_2$), 7.35 (2H, t, $J$ = 6.5 Hz, Ph 4-H), 7.39 (2H, t, $J$ = 6.5 Hz, Ph 3,5-$H_2$); MS *m/z* 291.1616 (M + H) ($C_{18}H_{19}N_4$ requires 291.1609), 199 (M – $C_7H_7$).

**1-(4-Chlorophenyl)-1-cyano-2-methoxybut-1-ene (99) and 2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine (117)**




(99)

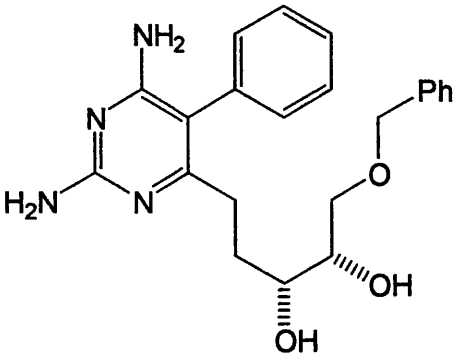



(117)

Compound (78) (1.50 g, 7.2 mmol) in diethyl ether (5 ml) was methylated with diazomethane as described before to afford (99) (1.40 g, 88%) as a pale yellow oil; NMR $\delta_H$ 1.32 (3H, t, $J$ = 7.6 Hz, $CH_2CH_3$), 2.80 (2H, q, $J$ = 7.6 Hz, $CH_2CH_3$), 3.88 (3H, s, $OCH_3$), 7.31 (2H, d, $J$ = 8.6 Hz,Ph 3,5-$H_2$), 7.61 (2H, d, $J$ = 8.6 Hz, Ph 2,6-$H_2$). A solution of sodium methoxide (0.56 g, 10 mmol) in 2-methoxyethanol (5 ml) was added to a solution of guanidine hydrochloride (1.0 g, 10 mmol) in 2-methoxyethanol (5 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (99) (1.52 g, 6.9 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (117) (0.86 g, 50%); $R_f$ = 0.42 as a white solid: mp 233-235 °C (lit.[110] mp 233-234 °C); NMR $\delta_H$ 0.97 (3H, t, $J$ = 7.4 Hz, $CH_3$), 2.09 (2H, q, $J$ = 7.4 Hz, $CH_2$), 5.64 (2H, br, $NH_2$), 5.92 (2H, br, $NH_2$), 7.22 (2H, d, $J$ = 8.2 Hz, Ph 3,5-$H_2$), 7.49 (2H, d, $J$ = 8.2 Hz, Ph 2,6-$H_2$); MS *m/z*

251.0884 (M + H) ($C_{12}H_{14}{}^{37}ClN_4$ requires 251.0877), 249.0909 (M + H) ($C_{12}H_{14}{}^{35}ClN_4$ requires 311.0910).

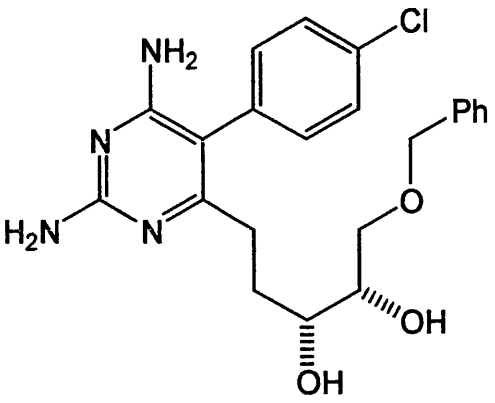
**2,4-Diamino-6-((3*R*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-phenylpyrimidine (118)**




**(118)**

The protected compound (100) (0.70 g, 1.6 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 70 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (118) (0.60 g, 95%) $R_f$ = 0.74 (chloroform/methanol, 2:1) as a very hygroscopic pale yellow solid; NMR ($CD_3OD$) $\delta_H$ 1.62-1.76 (1H, m, 2-H), 1.83-1.93 (1H, m, 2-H), 2.38-2.48 (1H, m, 1-H), 2.60 (1H, ddd, $J$ = 15.3, 10.6, 5.5 Hz, 1-H), 3.43-3.47 (2H, m, 3,4-$H_2$), 3.49-3.56 (2H, m, 5-$H_2$), 4.52 (1H, d, $J$ = 12.6 Hz, CHPh), 4.56 (1H, d, $J$ = 12.6 Hz, CHPh), 7.27-7.41 (6H, m, 2 × Ph 2,4,6-$H_3$), 7.47-7.58 (4H, m, 2 × Ph 3,5-$H_2$); MS *m/z* 395.2097 (M + H) ($C_{22}H_{27}N_4O_3$ requires 395.2083), 547 (M + 3-NOBA), 417 (M + Na), 243 (M - $C_9H_{11}O_2$), 213 (M – $C_{10}H_{13}O_3$), 91 (Bn).

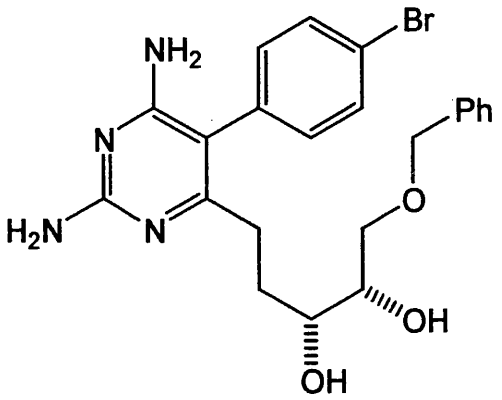
**2,4-Diamino-6-((3*R*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-(3-chlorophenyl)pyrimidine (119)**




**(119)**

The protected compound (101) (0.30 g, 0.7 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 30 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (119) (0.26 g, 87%) $R_f$ = 0.8 (chloroform/methanol, 2:1) as a white solid mp 131-133 °C; NMR ($CD_3OD$) $\delta_H$ 1.59-1.68 (1H, m, 2-H), 1.78-1.87 (1H, m, 2-H), 2.33-2.40 (1H, m, 1-H), 2.47-2.54 (1H, m, 1-H), 3.49-3.55 (2H, m, 3,4-$H_2$), 3.57-3.64 (2H, m, 5-$H_2$), 4.52 (1H, d, *J* = 12.5 Hz, CHPh), 4.56 (1H, d, *J* = 12.5 Hz, CHPh), 7.24 (2H, d, *J* = 8.6 Hz, Ph 3,5-$H_2$), 7.33-7.37 (5H, m, Ph-$H_5$), 7.47 (2H, d, *J* = 8.6 Hz, Ph 2,6-$H_2$); NMR ($CD_3OD$) $\delta_C$ 29.02 ($CH_2$), 30.84 ($CH_2$), 71.03 (5-$CH_2$), 71.61 (CH), 72.09 (CH), 72.99 (*C*-Ph), 107.42 (Pyr 5-C), 121.01 (Bn 1-C), 127.36 (2 × Bn CH), 127.58 (2 × Ph CH), 128.01 (2 × Ph CH), 129.32 (Bn 3,5-C), 132.16 (Ph 1-C), 134.14 (Ph 4-C), 138.10 (Bn CH), 161.81 (Pyr 2-C), 162.16 (Pyr 4-C), 163.66 (Pyr 6-C); MS *m/z* 431.1657 (M + H) ($C_{22}H_{26}{}^{37}ClN_4O_3$ requires 431.1663), 429.1680 (M + H) ($C_{22}H_{26}{}^{35}ClN_4O_4$ requires 429.1693), 453/451 (M + Na), 279/277 (M - $C_9H_{11}O_2$), 249/247 (M – $C_{10}H_{13}O_3$), 91 (Bn).

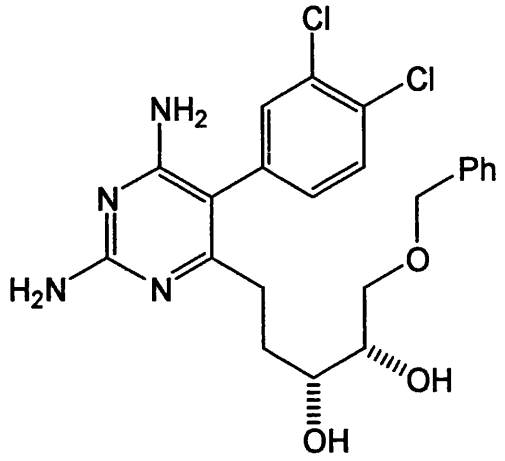
**2,4-Diamino-6-((3*R*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-(3-bromophenyl)pyrimidine (120)**




**(120)**

The protected compound (102) (1.10 g, 2.1 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 110 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (120) (0.90 g, 91%) $R_f$ = 0.66 (chloroform/methanol, 2:1) as a very hygroscopic buff solid; NMR $\delta_H$ 1.65-1.84 (2H, m, 2-$H_2$), 2.35-2.55 (2H, m, 1-$H_2$), 3.52-3.71 (4H, m, 3,4,5-$H_4$), 4.49 (1H, d, *J* = 12.1 Hz, CHPh), 4.54 (1H, d, *J* = 12.1 Hz, CHPh), 4.71 (2H, br, $NH_2$), 5.09 (2H, br, $NH_2$), 7.05 (2H, d, *J* = 8.4 Hz, Ph 3,5-$H_2$), 7.24-7.34 (5H, m, Ph-$H_5$), 7.54 (2H, d, *J* = 8.4 Hz, Ph 2,6-$H_2$); MS *m/z* 475.1184 (M + H) ($C_{22}H_{26}{}^{81}BrN_4O_3$ requires 475.1167), 473.1179 (M + H) ($C_{22}H_{26}{}^{79}BrN_4O_4$ requires 473.1188), 497/495 (M + Na), 323/321 (M - $C_9H_{11}O_2$), 293/291(M – $C_{10}H_{13}O_3$), 212 (M – $C_{10}H_{13}O_3Br$), 91 (Bn).

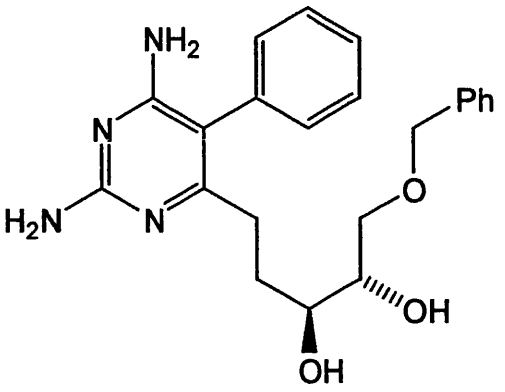
**2,4-Diamino-6-((3*R*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-(3,4-dichlorophenyl)pyrimidine (121)**




**(121)**

The protected compound (103) (0.34 g, 0.7 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 35 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (121) (0.24 g, 74%) $R_f$ = 0.66 (chloroform/methanol, 2:1) as a pale yellow solid mp 123-125 °C; NMR ($CD_3OD$) $\delta_H$ 1.53-1.67 (1H, m, 2-H), 1.74-1.90 (1H, m, 2-H), 2.31 (1H, ddd, *J* = 14.2, 9.0, 5.9 Hz, 1-H), 2.47 (1H, ddd, *J* = 14.2, 9.0, 5.9 Hz, 1-H), 3.44-3.51 (2H, m, 3,4-$H_2$), 3.54-3.59 (2H, m, 5-$H_2$), 4.47 (1H, d, *J* = 14.1 Hz, CHPh), 4.52 (1H, d, *J* = 14.1 Hz, CHPh), 7.15 (1H, dd, *J* = 8.2, 1.9 Hz, Ph 6-H), 7.22-7.33 (5H, m, Ph-$H_5$), 7.41 (1H, d, *J* = 1.9 Hz, Ph 2-H), 7.57 (1H, d, *J* = 8.2 Hz, Ph 5-H); NMR ($CD_3OD$) $\delta_C$ 23.30 ($CH_2$), 30.97 ($CH_2$), 71.23 and 71.58 (3,4-CH), 72.99 (5-$CH_2$ + *C*-Ph), 106.22 (Pyr 5-C), 181.16 (Bn 1-C), 127.31 (Ph CH), 127.55 (Ph CH), 127.99 (Ph CH), 130.64 (Ph C), 131.15 (Ph C), 131.95 (Ph 1-C), 132.61 (2 × Bn CH), 134.54 (2 × Bn CH), 138.17 (Bn CH), 162.09 (Pyr 2-C), 162.83 (Pyr 4-C), 163.16 (Pyr 6-C); MS *m/z* 467.1258 (M + H) ($C_{22}H_{25}{}^{37}Cl_2N_4O_3$ requires 467.1244), 465.1272 (M + H) ($C_{22}H_{25}{}^{37}Cl^{35}ClN_4O_3$ requires 465.1274), 463.1293 (M + H) ($C_{22}H_{25}{}^{35}Cl_2N_4O_3$ requires 463.1303), 489/487/485 (M + Na), 315/313/311 (M - $C_9H_{11}O_2$), 91 (Bn).

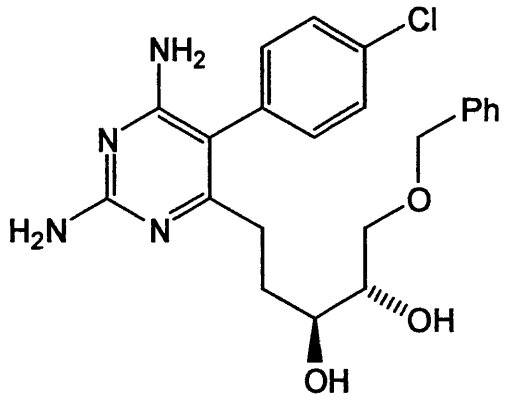
**2,4-Diamino-6-((3*S*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-phenylpyrimidine (122)**




**(122)**

The protected compound (104) (0.30 g, 0.7 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 30 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (122) (0.21 g, 76%); $R_f$ = 0.52 (chloroform/methanol, 2:1) as a buff solid: mp 101-102 °C; NMR ($CD_3OD$) $\delta_H$ 1.70 (1H, q, $J$ = 7.6 Hz, 2-$H_2$), 2.36 (1H, dt, $J$ = 14.2, 7.6 Hz, 1-H), 2.48 (1H, dt, $J$ = 14.2, 7.6 Hz, 1-H), 3.42-3.55 (4H, m, 3,4,5-$H_4$), 4.48 (1H, d, $J$ = 11.7 Hz, CHPh), 4.52 (1H, d, $J$ = 11.7 Hz, CHPh), 7.22-7.49 (10H, m, 2 × Ph-$H_5$); MS *m/z* 395.2082 (M + H) ($C_{22}H_{27}N_4O_3$ requires 359.2083), 91 (Bn).

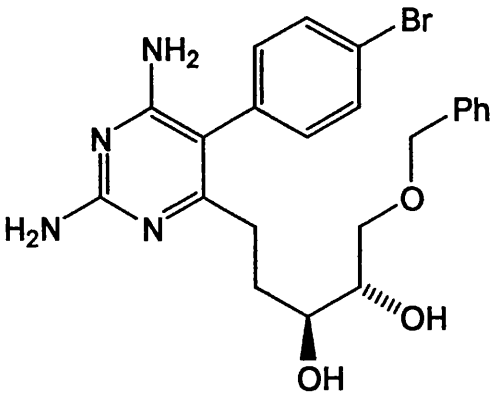
**2,4-Diamino-6-((3*S*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-(3-chlorophenyl)pyrimidine (123)**




**(123)**

The protected compound (87) (0.35 g, 0.75 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 35 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (123) (0.24 g, 75%); $R_f$ = 0.18 as a pale yellow solid: mp 141-143 °C; IR $\nu_{max}$ 3562 (OH), 3492, 3430, 3343 ($NH_2$), 1618 (C=N) $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 1.65-1.73 (2H, m, 2-$H_2$), 2.28 (1H, ddd, $J$ = 13.7, 9.4, 6.6 Hz, 1-H), 2.42 (1H, ddd, $J$ = 13.7, 9.4, 6.6 Hz, 1-H), 3.42-3.54 (4H, m, 3,4,5-$H_2$), 4.48 (1H, d, $J$ = 11.7 Hz, CHPh), 4.52 (1H, d, $J$ = 11.7 Hz, CHPh), 7.20 (2H, d, $J$ = 8.6 Hz, Ph 3,5-$H_2$), 7.30-7.36 (5H, m, Ph-$H_5$), 7.44 (2H, d, $J$ = 8.6 Hz, Ph 2,6-$H_2$); MS *m/z* 431.1680 (M + H) ($C_{22}H_{26}{}^{37}ClN_4O_3$ requires 431.1663), 429.1702 (M + H) ($C_{22}H_{26}{}^{35}ClN_4O_3$ requires 429.1693), 249/247 (M – $C_{10}H_{13}O_3$), 91 (Bn).

**2,4-Diamino-6-((3*S*,4*S*)-5-benzyloxy-3,4-dihydroxypentyl)-5-(3-bromophenyl)pyrimidine (124)**

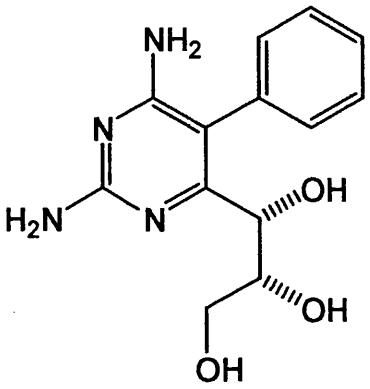



**(124)**

The protected compound (105) (0.20 g, 0.4 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 20 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (124) (0.18 g, 95%); $R_f$ = 0.82 (chloroform/methanol, 2:1) as a hygroscopic pale yellow solid; IR $\nu_{max}$ 3582 (OH), 3350 ($NH_2$), 1613 (C=N) $cm^{-1}$; NMR ($CD_3OD$) $\delta_H$ 1.71-1.78 (2H, m, 2-$H_2$), 2.36 (1H, dt, $J$ = 14.6, 7.8 Hz, 1-H), 2.46 (1H, dt, $J$ = 14.6, 7.8 Hz, 1-H), 3.43-3.61 (4H, m, 3,4,5-$H_4$), 4.51 (1H, d, $J$ = 11.9 Hz, CHPh), 4.56 (1H, d, $J$ = 11.9 Hz, CHPh), 7.18 (2H, d, $J$ = 8.6 Hz, Ph 3,5-$H_2$), 7.34-7.36 (5H, m, Ph-$H_5$), 7.63 (2H, d, $J$ = 8.6 Hz, Ph 2,6-$H_2$); NMR ($CD_3OD$) $\delta_C$ 29.26 ($CH_2$), 31.59 ($CH_2$), 70.80 and 72.32

(3,4-C), 71.11 (5-C), 72.99 (*C*-Ph), 107.30 (Pyr 5-C), 120.98 (Ph 4-C), 122.10 (Bn 1-C), 127.39 (2 × Bn CH), 127.58 (2 × Ph CH), 128.04 (2 × Ph CH), 130.83 (Ph 1-C), 132.31 (2 × Bn CH), 138.05 (Bn CH), 161.45 (Pyr 2-C), 161.80 (Pyr 4-C), 162.50 (Pyr 6-C); MS *m/z* 475.1184 (M + H) ($C_{22}H_{26}{}^{81}BrN_4O_3$ requires 475.1167), 473.1186 (M + H) ($C_{22}H_{26}{}^{79}BrN_4O_4$ requires 473.1188), 323/321 (M – $C_9H_{11}O_2$), 91 (Bn).

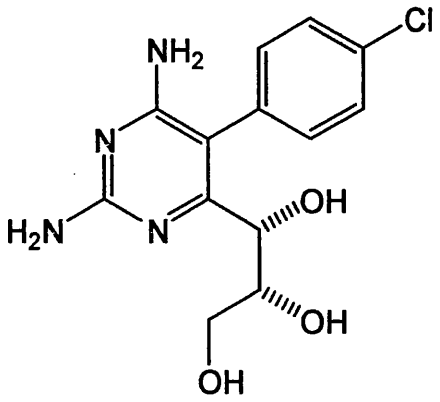
**2,4-Diamino-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)-5-phenylpyrimidine (125)**




**(125)**

The protected compound (111) (0.35 g, 1.1 mmol) was stirred for 2 h in aqueous trifluoroacetic acid (30%, 35 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (125) (0.22 g, 73%); $R_f$ = 0.16 (chloroform/methanol, 2:1); $[\alpha]^{20}{}_D$ = -0.38° (c 4, $CH_3OH$) as a hygroscopic white solid; NMR ($CD_3CN$) $\delta_H$ 3.45 (1H, dd, *J* = 11.6, 4.9 Hz, 3-H), 3.48 (1H, dd, *J* = 11.6, 3.9 Hz, 3-H), 3.70-3.73 (1H, m, 2-H), 4.46 (1H, d, *J* = 6.2 Hz, 1-H), 4.73 (2H, br, OH/NH), 5.82 (1H, br, OH/NH), 6.98 (1H, br, OH/NH), 7.31 (2H, dd, *J* = 7.4, 2.0 Hz, Ph 2,6-$H_2$), 7.45-7.61 (3H, m, Ph 3,4,5-$H_3$); MS *m/z* 277.1308 (M + H) ($C_{13}H_{17}N_4O_3$ requires 277.1300), 299 (M + Na), 215 (M – $C_2H_5O_2$).

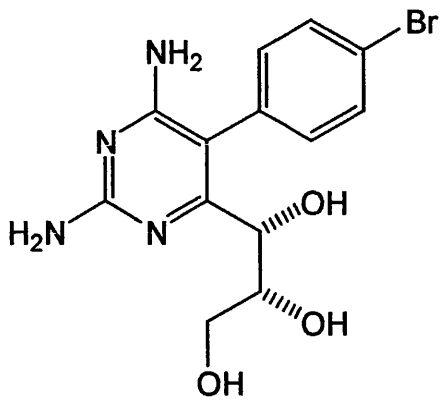
**2,4-Diamino-5-(4-chlorophenyl)-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine (126)**




**(126)**

The protected compound (112) (0.15 g, 0.43 mmol) was stirred overnight in aqueous trifluoroacetic acid (30%, 15 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (126) (0.12 g, 90%); $R_f$ = 0.17 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = -41° (c 0.4, $CH_3OH$) as a pale yellow solid: mp 196-197 °C; IR $\nu_{max}$ 3550 (OH), 3475, 3413 ($NH_2$), 1617 (C=N) $cm^{-1}$; NMR ($(CD_3)_2CO$) $\delta_H$ 3.58 (1H, dd, *J* = 11.3, 4.7 Hz, 3-H), 3.63 (1H, dd, *J* = 11.3, 3.5 Hz, 3-H), 3.89-3.92 (1H, m, 2-H), 4.54 (1H, d, *J* = 7.0 Hz, 1-H), 5.54 (5H, br, OH/NH), 6.62 (1H, br, OH/NH), 7.40 (2H, d, *J* = 7.4 Hz, Ph 3,5-$H_2$), 7.47 (2H, d, *J* = 7.4 Hz, Ph 2,6-$H_2$), 7.79 (1H, br, OH/NH); NMR ($CD_3CN$) $\delta_C$ 62.60 ($CH_2$), 69.31 (CH), 72.47 (CH), 108.42 (Pyr 5-C), 129.88 (2 × Ph CH), 132.39 (2 × Ph CH), 133.53 (Ph 1-C), 134.72 (Ph 4-C), 161.21 (Pyr 2-C), 161.53 (Pyr 4-C), 161.87 (Pyr 6-C); MS *m/z* 313.0877 (M + H) ($C_{13}H_{16}{}^{37}ClN_4O_3$ requires 313.0881), 311.0905 (M + H) ($C_{13}H_{16}{}^{35}ClN_4O_3$ requires 311.0910).

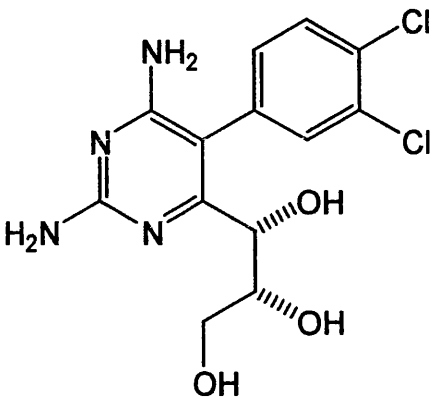
**2,4-Diamino-5-(4-bromophenyl)-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine (127)**




**(127)**

The protected compound (113) (0.40 g, 1.0 mmol) was stirred for 4 h in aqueous trifluoroacetic acid (30%, 40 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (127) (0.35 g, 95%); $R_f$ = 0.18 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = -15° (c 0.94, $CH_3OH$) as a hygroscopic pale yellow solid; IR $\nu_{max}$ 3550 (OH), 3478, 3414 ($NH_2$), 1618 (C=N) $cm^{-1}$; NMR ($CD_3CN$) $\delta_H$ 3.51 (1H, dd, $J$ = 12.3, 4.9 Hz, 3-H), 3.55 (1H, dd, $J$ = 12.3, 4.5 Hz, 3-H), 3.72-3.76 (1H, m, 2-H), 4.49 (1H, d, $J$ = 6.2 Hz, 1-H), 5.94 (1H, br, OH/NH), 7.03 (1H, br, OH/NH), 7.29 (2H, d, $J$ = 8.0 Hz, Ph 3,5-$H_2$), 7.71 (2H, d, $J$ = 8.0 Hz, Ph 2,6-$H_2$); NMR ($CD_3CN$) $\delta_C$ 62.47 ($CH_2$), 68.98 (CH), 72.30 (CH), 108.83 (Pyr 5-C), 123.37 (Ph 4-C), 129.70 (Ph 1-C), 132.92 (Ph CH), 132.98 (Ph CH), 133.81 (Ph CH), 156.05 (Pyr 2-C), 162.06 (Pyr 4-C), 164.91 (Pyr 6-C); MS *m/z* 357.0396 (M + H) ($C_{13}H_{16}{}^{81}BrN_4O_3$ requires 357.0385), 355.0412 (M + H) ($C_{13}H_{16}{}^{79}BrN_4O_3$ requires 355.0405), 379/377 (M + Na), 295/293 (M – $C_2H_5O_2$).

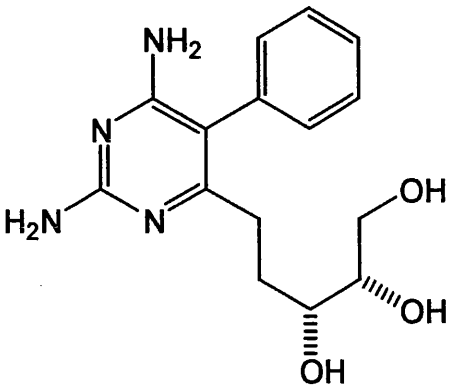
**2,4-Diamino-5-(3,4-dichlorophenyl)-6-((1*S*,2*R*)-1,2,3-trihydroxypropyl)pyrimidine (128)**




**(128)**

The protected compound (114) (0.20 g, 0.5 mmol) was stirred for 4 h in aqueous trifluoroacetic acid (30%, 20 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (128) (0.15 g, 87%); $R_f$ = 0.34 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = -3° (c 4.7, $CH_3OH$) as a pale yellow solid: mp 120-121 °C; IR $\nu_{max}$ 3549 (OH), 3476, 3415 ($NH_2$), 1618 (C=N) $cm^{-1}$; NMR ($CD_3CN$) $\delta_H$ 3.40-3.46 (2H, m, 3-$H_2$), 3.63-3.68 (1H, m, 2-H), 4.35 (1H, d, $J$ = 4.7 Hz, 1-H), 5.25 (2H, br, $NH_2$), 5.67 (2H, br, $NH_2$), 7.20 (1H, dd, $J$ = 8.0, 1.9 Hz, Ph 6-H), 7.46 (1H, d, $J$ = 1.9 Hz, Ph 2-H), 7.60 (1H, d, $J$ = 8.0 Hz, Ph 5-H); NMR ($(CD_3)_2SO$) $\delta_C$ 63.67 ($CH_2$), 69.38 (CH), 74.29 (CH), 106.16 (Pyr 5-C), 130.30 (Ph C), 131.08 (Ph CH), 131.44 (Ph C), 131.63 (Ph CH), 132.68 (Ph CH), 134.09 (Ph C), 162.01 (Pyr 2-C), 162.81 (Pyr 4-C), 164.00 (Pyr 6-C); MS *m/z* 349.0469 (M + H) ($C_{13}H_{15}{}^{37}Cl_2N_4O_3$ requires 349.0462), 347.0501 (M + H) ($C_{13}H_{15}{}^{37}Cl^{35}ClN_4O_3$ requires 347.0491), 345.0521 (M + H) ($C_{13}H_{15}{}^{35}Cl_2N_4O_3$ requires 345.0521), 371/369/367 (M + Na), 287/285/283 (M – $C_2H_5O_2$).

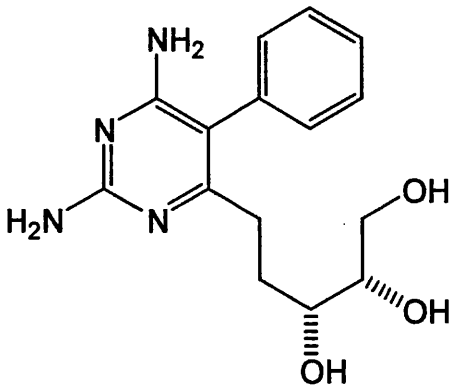
**2,4-Diamino-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)-5-phenylpyrimidine (129)**




**(129)**

Ammonia (10 ml) was distilled and allowed to warm to reflux (-33 °C), and sodium (84 mg, 3.6 mmol), was added until a deep blue solution was sustained. Compound (118) (150 mg, 0.4 mmol) in THF (5 ml) was added directly to the Na / $NH_3$ solution. After being stirred for 20 min at reflux, the reaction was quenched with saturated aqueous ammonium chloride (2 ml) and then allowed to warm to room temperature. After evaporation to dryness, a mixture of chloroform and methanol (2:1, 20 ml) was added followed by filtration. The residue remaining after evaporation of the organic layer was purified by chromatography (chloroform/methanol, 7:3) to afford (129) (90 mg, 78%) $R_f$ = 0.13 (chloroform/methanol, 2:1) as a white solid: mp 90-91 °C; NMR ($D_2O$) $\delta_H$ 1.29-1.36 (1H, m, 2-H), 1.49-1.55 (1H, m, 2-H), 2.07 (1H, ddd, $J$ = 13.0, 10.2, 6.2 Hz, 1-H), 2.21 (1H, ddd, $J$ = 13.0, 10.5, 5.3 Hz, 1-H), 3.17-3.21 (1H, m, 3-H), 3.22-3.25 (2H, m, 5-$H_2$), 3.37 (1H, dt, $J$ = 8.5, 6.1 Hz, 4-H), 7.03 (1H, d, $J$ = 7.5 Hz, Ph 2-H), 7.04 (1H, d, $J$ = 7.5 Hz, Ph 6-H), 7.23 (1H, t, $J$ = 7.5 Hz, Ph 4-H), 7.29 (2H, t, $J$ = 7.5 Hz, Ph 3,5-$H_2$); NMR ($D_2O$) $\delta_C$ 30.24 ($CH_2$), 31.10 ($CH_2$), 62.25 (5-$CH_2$), 71.35 (CH), 74.11 (CH), 109.19 (Pyr 5-C), 128.08 (Ph CH), 129.19 (2 × Ph CH), 130.54 (2 × Ph CH), 133.88 (Ph 1-C), 161.15 (Pyr 2-C), 162.92 (Pyr 4-C), 165.94 (Pyr 6-C); MS *m/z* 305.1616 (M + H) ($C_{15}H_{21}N_4O_3$ requires 305.1613), 327 (M + Na), 243 (M - $C_2H_5O_2$), 213 (M – $C_3H_7O_3$).

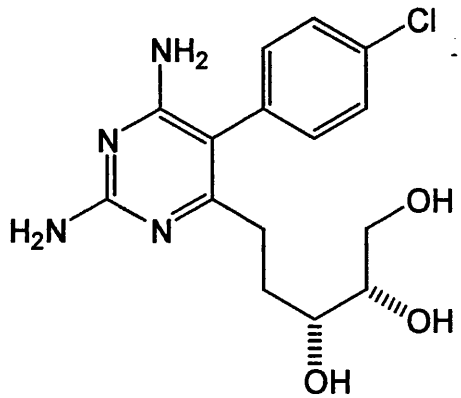
**2,4-Diamino-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)-5-phenylpyrimidine (129)**



**(129)**

The protected compound (144) (0.20 g, 0.58 mmol) was stirred for 6 h in aqueous trifluoroacetic acid (30%, 20 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (129) (0.15 g, 85%); $R_f$ = 0.13 (chloroform/methanol, 2:1) as a white solid: mp 90-91 °C; MS *m/z* 305.1620 (M + H) ($C_{15}H_{21}N_4O_3$ requires 305.1613), 327 (M + Na), 243 (M – $C_2H_5O_2$), 213 (M – $C_3H_7O_3$); NMR spectra are as described before.

**2,4-Diamino-5-(4-chlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (130)**

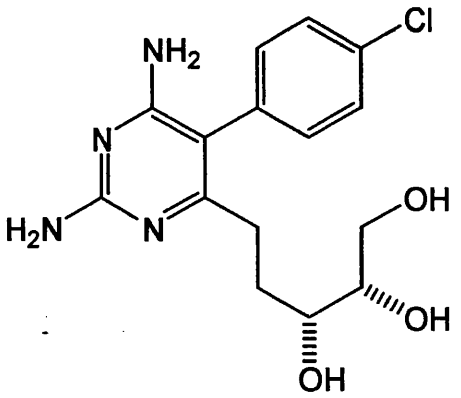


**(130)**

Anhydrous iron (III) chloride (68 mg, 0.42 mmol) was added to a solution of compound (119) (60 mg, 0.14 mmol) in anhydrous dichloromethane (5 ml) under nitrogen. After 80 min the reaction was quenched by addition of water (2 ml). The

residue remaining after evaporation of the solvent was purified by chromatography (chloroform/methanol, 7:3) to afford (130) (30 mg, 63%); $R_f$ = 0.11 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = -1.0° (c 1.1, $CH_3OH$) as a white solid: mp 250-251 °C; NMR ($D_2O$) $\delta_H$ 1.40-1.49 (1H, m, 2-H), 1.61-1.68 (1H, m, 2-H), 2.22 (1H, ddd, $J$ = 13.6, 10.4, 6.0 Hz, 1-H), 2.36 (1H, ddd, $J$ = 13.6, 10.0, 5.2 Hz, 1-H), 3.29-3.33 (1H, m, 3-H), 3.35-3.38 (2H, m, 5-$H_2$), 3.48-3.53 (1H, m, 4-H), 7.18 (2H, d, $J$ = 8.6 Hz, Ph 3,5-$H_2$), 7.44 (2H, d, $J$ = 8.5 Hz, Ph 2,6-$H_2$); NMR ($CD_3OD$) $\delta_C$ 30.05 ($CH_2$), 31.36 ($CH_2$), 62.70 (5-$CH_2$), 71.97 (CH), 74.14 (CH), 107.03 (Pyr 5-C), 129.18 (2 × Ph CH), 132.23 (2 × Ph CH), 133.13 (Ph C), 133.71 (Ph C), 161.73 (Pyr 2-C), 162.08 (Pyr 4-C), 163.06 (Pyr 6-C); MS *m/z* 341.1185 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_3$ requires 341.1194), 339.1217 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_3$ requires 339.1223), 308/306 (M - $CH_4O$), 249/247 (M – $C_3H_7O_3$).

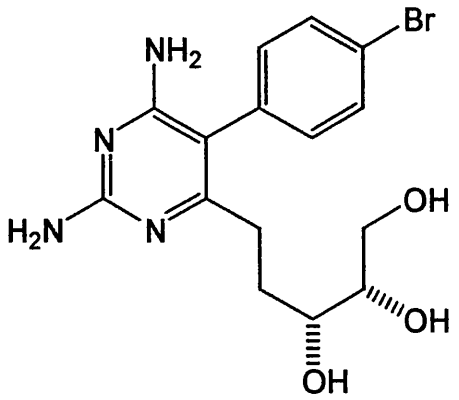
**2,4-Diamino-5-(4-chlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (130)**




**(130)**

The protected compound (145) (50 mg, 0.13 mmol) was stirred for 6 h in aqueous trifluoroacetic acid (30%, 5 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (130) (40 mg, 91%); $R_f$ = 0.11 (chloroform/methanol, 2:1) as a white solid: mp 250-251 °C; MS *m/z* 341.1200 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_3$ requires 341.1194), 339.1225 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_3$ requires 339.1223), 308/306 (M - $CH_4O$), 249/247 (M – $C_3H_7O_3$), 212 (M - $C_3H_7ClO_3$); NMR spectra are as described before.

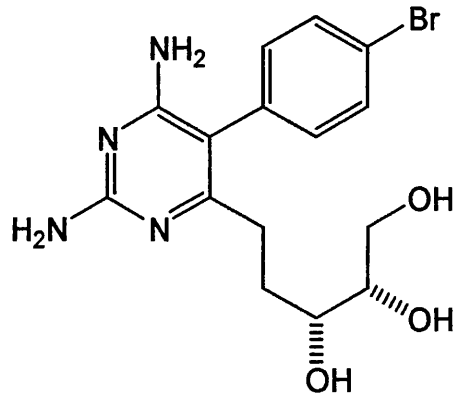
**2,4-Diamino-5-(4-bromophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (131)**




**(131)**

The protected compound (120) (40 mg, 0.09 mmol) was subjected to reaction with anhydrous iron (III) chloride (44 mg, 0.27 mmol) as described for compound (131) to afford (24) (22 mg, 85%); $R_f$ = 0.18 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = -4.2° (c 0.24, $CH_3OH$) as a white solid: mp 198-200 °C; NMR ($D_2O$) $\delta_H$ 1.40-1.54 (1H, m, 2-H), 1.75-1.87 (1H, m, 2-H), 2.13-2.24 (1H, m, 1-H), 2.28-2.38 (1H, m, 1-H), 3.24-3.34 (1H, m, 3-H), 3.32-3.36 (2H, m, 5-$H_2$), 3.44-3.52 (1H, m, 4-H), 7.08 (2H, d, *J* = 8.0 Hz, Ph 3,5-$H_2$), 7.55 (2H, d, *J* = 8.0 Hz, Ph 2,6-$H_2$); MS *m/z* 385.0683 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O_3$ requires 385.0698).

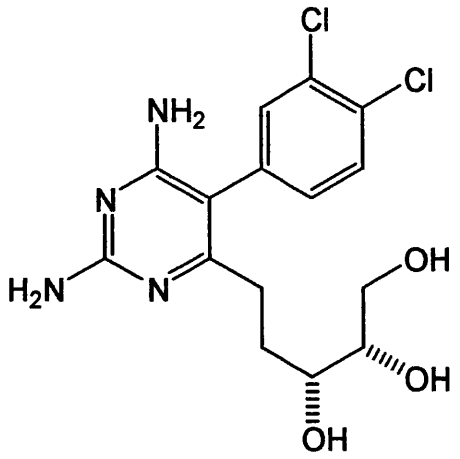
**2,4-Diamino-5-(4-bromophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (131)**




**(131)**

The protected compound (146) (40 mg, 0.09 mmol) was stirred for 6 h in aqueous trifluoroacetic acid (30%, 4 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (131) (30 mg, 87%); $R_f$ = 0.18 (chloroform/methanol, 2:1) as a pale yellow solid: mp 198-200 °C; MS *m/z* 385.0702 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O_3$ requires 385.0698), 383.0714 (M + H) ($C_{15}H_{20}{}^{79}BrN_4O_3$ requires 383.0718); NMR spectra are as described before.

**2,4-Diamino-5-(3,4-dichlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (132)**

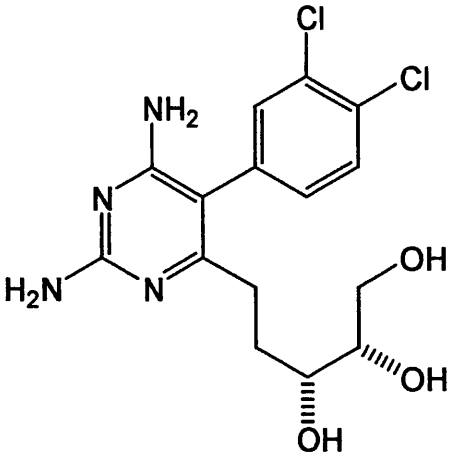



**(132)**

The protected compound (121) (60 mg, 0.14 mmol) was subjected to reaction with anhydrous iron (III) chloride (65 mg, 0.42 mmol) as described for deprotection of compound (130) to afford (132) (40 mg, 77%) $R_f$ = 0.13 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = -1.4° (c 2.2, $CH_3OH$) as a white solid: mp 180-181 °C; NMR ($(CD_3)_2SO$) $\delta_H$ 1.38-1.48 (1H, m, 2-H), 1.57-1.68 (1H, m, 2-H), 2.08 (1H, ddd, *J* = 13.6, 10.4, 5.5 Hz, 1-H), 2.34 (1H, ddd, *J* = 13.6, 10.4, 5.5 Hz, 1-H), 3.14-3.20 (1H, m, 3-H), 3.28-3.31 (2H, m, 5-$H_2$), 3.46-3.49 (1H, m, 4-H), 5.79 (2H, br, $NH_2$), 5.97 (1H, br, $NH_2$), 7.16 (1H, dd, *J* = 8.2, 1.8 Hz, Ph 6-H), 7.42 (1H, d, *J* = 1.8 Hz, Ph 2-H), 7.66 (1H, d, *J* = 8.2 Hz, Ph 5-H); NMR ($(CD_3)_2SO$) $\delta_C$ 31.26 ($CH_2$), 32.09 ($CH_2$), 63.94 (5-$CH_2$), 71.95 (CH), 75.19 (CH), 105.22 (Pyr 5-C), 131.38 (Ph CH), 131.81 (Ph CH), 133.16 (Ph CH), 133.74 (Ph C), 133.97 (Ph C), 137.51 (Ph C), 162.42 (Pyr 2,4-C), 166.19 (Pyr 6-C); MS *m/z* 377.0812 (M + H) ($C_{15}H_{19}{}^{37}Cl_2N_4O_3$ requires 377.0775),

375.0814 (M + H) ($C_{15}H_{19}{}^{37}Cl\ {}^{35}ClN_4O_3$ requires 375.0804), 373.0836 (M + H) ($C_{15}H_{19}{}^{35}Cl_2N_4O_3$ requires 373.0834), 345/343/341 (M - $CH_3O$).

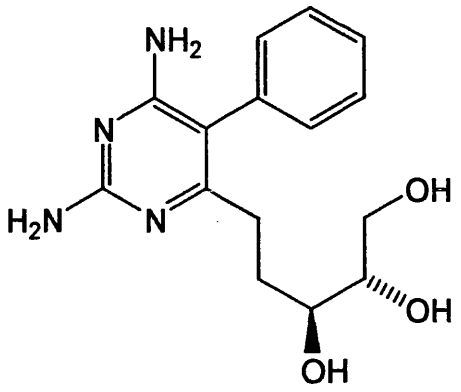
**2,4-Diamino-5-(3,4-dichlorophenyl)-6-((3*R*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (132)**




**(132)**

The protected compound (147) (30 mg, 0.07 mmol) was stirred for 6 h in aqueous trifluoroacetic acid (30%, 3 ml). The solvent was evaporated and the residue was purified by chromatography (chloroform/methanol, 7:3) to give (132) (20 mg, 77%); $R_f$ = 0.13 (chloroform/methanol, 2:1) as a white solid: mp 180-181 °C; MS *m/z* 377.0794 (M + H) ($C_{15}H_{19}{}^{37}Cl_2N_4O_3$ requires 377.0775), 375.0820 (M + H) ($C_{15}H_{19}{}^{37}Cl\ {}^{35}ClN_4O_3$ requires 375.0804), 373.0838 (M + H) ($C_{15}H_{19}{}^{35}Cl_2N_4O_3$ requires 373.0834), 315/313/311 (M – $C_2H_5O_2$), 212 (M - $C_3H_6Cl_2O_3$); NMR spectra are as described before.

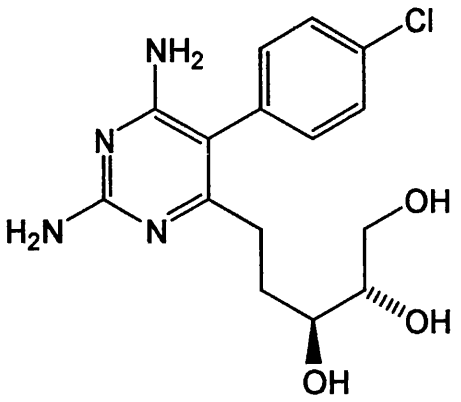
**2,4-Diamino-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)-5-phenylpyrimidine (133)**




**(133)**

The protected compound (122) (0.20 g, 0.5 mmol) was stirred in methanol (20 ml) with Pd/C (5%, 154 mg) and 5 drops of chloroform under atmosphere of hydrogen for 2 h. the catalyst was removed by filtration through Celite®. The solvent was evaporated off and the residue was purified by chromatography (chloroform/methanol, 7:3) to afford (133) (150 mg, 93%); $R_f$ = 0.05 as a white solid mp >350 °C; $[\alpha]^{20}_D$ = +10.9° (c 0.5, $H_2O$); NMR ($D_2O$) $\delta_H$ 1.50-1.64 (2H, m, 2-$H_2$), 2.28 (1H, ddd, *J* = 13.9, 10.1, 6.3 Hz, 1-H), 2.40 (1H, ddd, *J* = 13.9, 10.1, 6.3 Hz, 1-H), 3.31-3.45 (4H, m, 3,4,5-$H_4$), 7.25 (2H, d, *J* = 7.0 Hz, Ph 3,5-$H_2$), 7.42 (1H, t, *J* = 7.0 Hz, Ph 4-H), 7.47 (2H, t, *J* = 7.0 Hz, Ph 2,6-$H_2$); NMR ($D_2O$) $\delta_C$ 29.75 ($CH_2$), 31.57 ($CH_2$), 62.63 (5-$CH_2$), 73.61 and 73.64 (3,4-C), 109.24 (Pyr 5-C), 128.37 (Ph CH), 129.37 (2 × Ph CH), 130.56 (2 × Ph CH), 133.41 (Ph 1-C), 160.29 (Pyr 2-C), 161.05 (Pyr 4-C), 161.81 (Pyr 6-C); MS *m/z* 305.1618 (M + H) ($C_{15}H_{21}N_4O_3$ requires 305.1613), 192 (M – $C_6H_8O_2$).

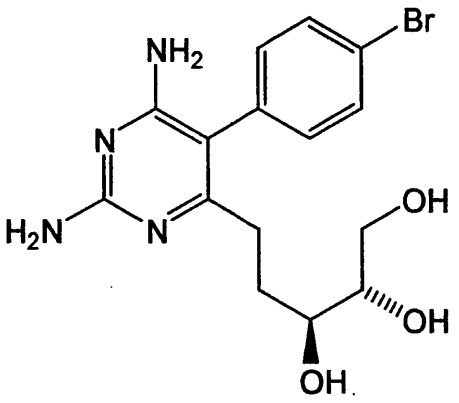
**2,4-Diamino-5-(4-chlorophenyl)-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (134)**




**(134)**

Anhydrous iron (III) chloride (65 mg, 0.4 mmol) was added to a solution of compound (123) (60 mg, 0.14 mmol) in anhydrous dichloromethane (5 ml) under nitrogen. After 80 min the reaction was quenched by addition of water (2 ml). The residue remaining after evaporation of the solvent was purified by chromatography (chloroform/methanol, 7:3) to afford (134) (40 mg, 77%); $R_f$ = 0.13 (chloroform/methanol, 2:1); $[\alpha]^{20}_D$ = +6.0° (c 0.67, $H_2O$) as a white solid mp >350 °C; NMR ($CD_3OD$) $\delta_H$ 1.73-1.77 (2H, m, 2-$H_2$), 2.41 (1H, dt, $J$ = 14.4, 6.7 Hz, 1-H), 2.54 (1H, dt, $J$ = 14.4, 6.7 Hz, 1-H), 3.44-3.61 (4H, m, 3,4,5-$H_4$), 7.31 (2H, d, $J$ = 8.4 Hz, Ph 3,5-$H_2$), 7.53 (2H, d, $J$ = 8.4 Hz, Ph 2,6-$H_2$); NMR ($CD_3OD$) $\delta_C$ 27.08 ($CH_2$), 31.39 ($CH_2$), 62.91 (5-$CH_2$), 70.52 and 73.58 (3,4-C), 108.29 (Pyr 5-C), 129.60 (2 × Ph CH), 132.04 (2 × Ph CH), 137.45 (Ph 1-C), 137.50 (Ph 4-C), 156.86 (Pyr 2-C), 157.38 (Pyr 4-C), 157.80 (Pyr 6-C); MS *m/z* 341.1180 (M + H) ($C_{15}H_{20}{}^{37}ClN_4O_3$ requires 341.1194), 339.1237 (M + H) ($C_{15}H_{20}{}^{35}ClN_4O_3$ requires 339.1223), 303 (M - Cl).

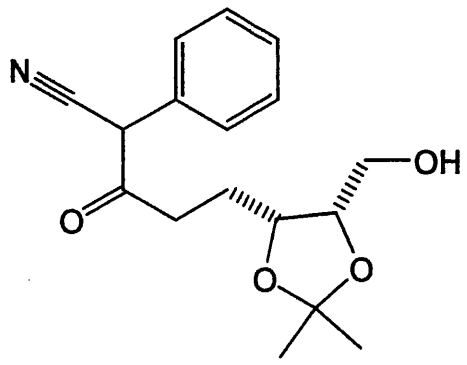
**2,4-Diamino-5-(4-bromophenyl)-6-((3*S*,4*S*)-3,4,5-trihydroxypentyl)pyrimidine (135)**




**(135)**

The protected compound (124) (80 mg, 0.17 mmol) was subjected to reaction with anhydrous iron (III) chloride (83 mg, 0.5 mmol) as described for deprotection of compound (134) to afford (135) (50 mg, 77%); $R_f$ = 0.08; $[\alpha]^{20}_D$ = +12.5° (c 0.24, $CH_3OH$) as a white solid: mp >350 °C; IR $\nu_{max}$ 3649 (OH), 3468, 3418 ($NH_2$), 1618 (C=N) $cm^{-1}$; NMR ($D_2O$) $\delta_H$ 1.60-1.72 (2H, m, 2-$H_2$), 2.30-2.54 (2H, m, 1-$H_2$), 3.42-3.56 (4H, m, 3,4,5-$H_4$), 7.25 (2H, d, *J* = 8.7 Hz, Ph 3,5-$H_2$), 7.72 (2H, d, *J* = 8.7 Hz, Ph 2,6-$H_2$); MS *m/z* 385.0707 (M + H) ($C_{15}H_{20}{}^{81}BrN_4O_3$ requires 385.0698), 383.0717 (M + H) ($C_{15}H_{20}{}^{79}BrN_4O_3$ requires 383.0718), 241 (M - $C_2H_6BrO_2$).

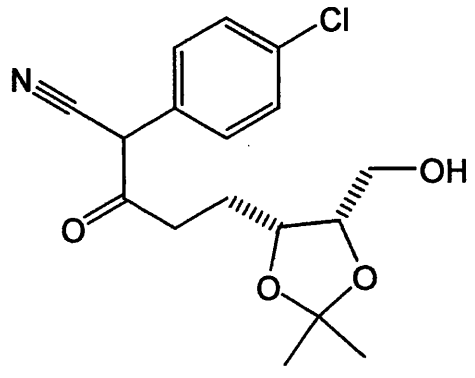
**(4*R*,5*S*)-4-(4-Cyano-3-oxo-4-phenylbutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (136)**




**(136)**

Lithium bis(trimethylsilyl)amide (1.0 M in THF) (30 ml, 30 mmol) was added to phenylacetonitrile (1.75 g, 15 mmol) in dry diethyl ether (15 ml) under nitrogen at -78 °C. After 10 min, compound (47) was added and the reaction mixture was allowed to warm to room temperature. The mixture was stirred for 72 h. An excess of diethyl ether was added, followed by water. The aqueous layer was separated, washed with diethyl ether and then added to ethyl acetate (100 ml) in a separating funnel. Acidification by dropwise addition of 1 M aqueous citric acid to pH = 6 with rapid extraction with ethyl acetate was followed by rapid separation of the organic layer. Ethyl acetate was washed with water, dried ($MgSO_4$) and evaporated. Purification with chromatography (ethyl acetate/hexane, 3:1) afforded (136) (0.45 g, 10%); $R_f$ = 0.37 as a hygroscopic pale yellow solid; NMR $\delta_H$ 1.30 (3H, s, Me), 1.40 (3H, s, Me), 1.74-1.81 (2H, m, C*H*$_2$CHO), 2.66-2.76 (1H, m, C*H*CH$_2$), 2.78-2.88 (1H, m, C*H*CH$_2$), 3.62 (2H, d, $J$ = 5.5 Hz, $CH_2OH$), 4.03-4.08 (1H, m, 4-H), 4.12-4.17 (1H, m, 5-H), 4.79 (1H, s, CHCN), 7.36-7.50 (5H, m, Ph-H$_5$); MS *m/z* 303.1464 (M + H) ($C_{17}H_{21}NO_4$ requires 303.1470), 287 (M - $CH_3$), 271 (M - $CH_3O$), 245 (M – $C_2H_3NO$).

**(4*R*,5*S*)-4-(4-(4-Chlorophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (137)**

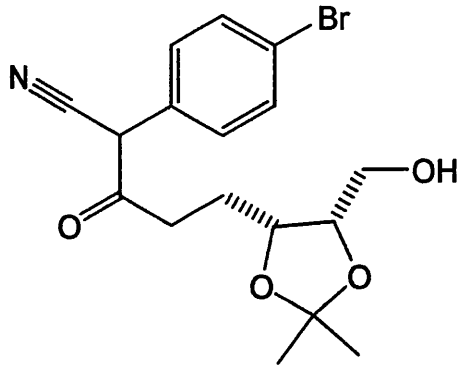



**(137)**

4-Chlorophenylacetonitrile (2.3 g, 15 mmol) and compound (47) (3.5 g, 15 mmol) were condensed in the same method as described above. The resulting residue was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (137) (0.35 g, 7%);

$R_f$ = 0.26 as a pale yellow solid: mp 133-135 °C; NMR $\delta_H$ 1.42 (3H, s, Me), 1.52 (3H, s, Me), 2.35-2.52 (2H, m, C$H_2$CHO), 2.66-2.84 (2H, m, C$H_2$CH$_2$), 3.63 (1H, dd, $J$ = 12.7, 4.9 Hz, C$H$OH), 3.73 (1H, dd, $J$ = 12.7, 4.9 Hz, C$H$OH), 4.21-4.28 (2H, m, 4,5-H$_2$), 5.53 (0.2H, s, CHN), 7.44 (2H, d, $J$ = 9.0 Hz, Ph 3,5-H$_2$), 7.63 (0.4H, d, $J$ = 8.6 Hz, Ph 2,6-H$_2$), 8.01 (1.6H, d, $J$ = 9.0 Hz, Ph 2,6-H$_2$); MS *m/z* 340 (M + H) $C_{17}H_{20}{}^{37}ClNO_4$, 338 (M + H) $C_{17}H_{20}{}^{35}ClNO_4$, 322/320 (M – OH), 308/306 (M - $CH_3O$), 298/296 (M – $C_2H_3N$).

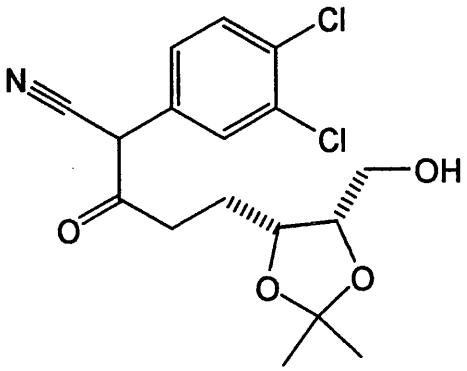
**(4*R*,5*S*)-4-(4-(4-Bromophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (138)**




**(138)**

4-Bromophenylacetonitrile (2.9 g, 15 mmol) and compound (47) (3.5 g, 15 mmol) were condensed in the same method as described above. The resulting residue was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (138) (0.32 g, 6%); $R_f$ = 0.21 as a pale yellow solid: mp 128-130 °C; NMR $\delta_H$ 1.41 (3H, s, Me), 1.51 (3H, s, Me), 2.39-2.48 (2H, m, C$H_2$CHO), 2.69-2.79 (2H, m, C$H_2$CH$_2$), 3.62 (1H, dd, $J$ = 11.6, 5.3 Hz, C$H$OH), 3.73 (1H, dd, $J$ = 11.6, 5.3 Hz, C$H$OH), 4.19-4.27 (2H, m, 4,5-H$_2$), 7.30 (2H, d, $J$ = 8.5 Hz, Ph 2,6-H$_2$), 7.47 (2H, d, $J$ = 8.5 Hz, Ph 3,5-H$_2$); MS *m/z* 382.0480 (M + H) ($C_{17}H_{20}{}^{81}BrNO_4$ requires 382.0476), 380.0475 (M + H) ($C_{17}H_{20}{}^{79}BrNO_4$ requires 380.0497), 368/366 (M - $CH_3$), 342/340 (M - $C_2H_3N$), 326/324 (M – $C_2H_3NO$).

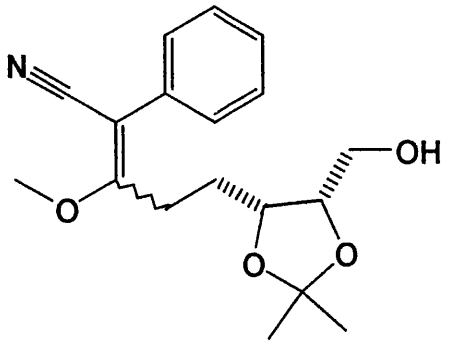
**(4*R*,5*S*)-4-(4-(3,4-Dichlorophenyl)-4-cyano-3-oxobutyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (139)**



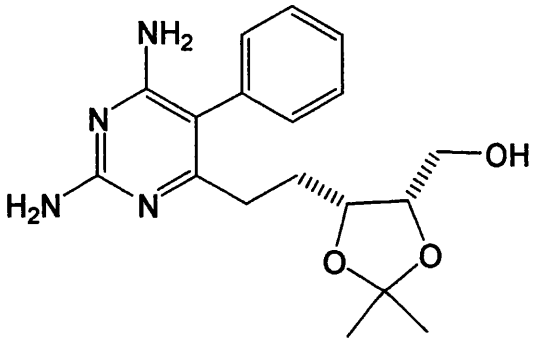

**(139)**

3,4-Dichlorophenylacetonitrile (2.8 g, 15 mmol) and compound (47) (3.5 g, 15 mmol) were condensed in the same method as described above. The resulting residue was purified by chromatography (ethyl acetate/hexane, 3:1) to afford (139) (0.40 g, 7%); $R_f$ = 0.37 as a pale yellow solid: mp 117-118 °C; IR $\nu_{max}$ 3424 (OH), 2209 (CN), 1718 (C=O) $cm^{-1}$; NMR $\delta_H$ 1.42 (3H, s, Me), 1.52 (3H, s, Me), 2.38-2.47 (2H, m, C*H*$_2$CHO), 2.72-2.84 (2H, m, C*H*$_2$CH$_2$), 3.64 (1H, dd, *J* = 11.5, 5.5 Hz, C*H*OH), 3.75 (1H, dd, *J* = 11.5, 5.5 Hz, C*H*OH), 4.18-4.29 (2H, m, 4,5-H$_2$), 7.27 (1H, dd, *J* = 8.5, 2.2 Hz, Ph 6-H), 7.43 (1H, d, *J* = 8.5 Hz, Ph 5-H), 7.54 (1H, d, *J* = 2.2 Hz, Ph 2-H). MS *m/z* 375 (M + H) $C_{17}H_{19}{}^{37}Cl_2NO_4$, 373 (M + H) $C_{17}H_{19}{}^{37}Cl^{35}ClNO_4$, 371 (M + H) $C_{17}H_{19}{}^{35}Cl_2NO_4$, 358/356/354 (M – $CH_4$), 334/332/330 (M - $C_2H_2N$), 316/314/312 (M – $C_2H_3NO$).

**(4*R*,5*S*)-4-(4-Cyano-3-methoxy-4-phenylbut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (140) and 2,4-diamino-6-(2-((4*R*,5*S*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)-5-phenylpyrimidine (144)**




(140)

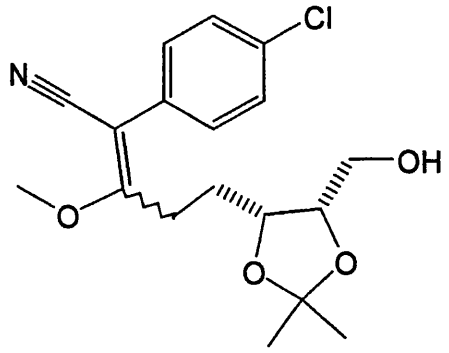



(144)

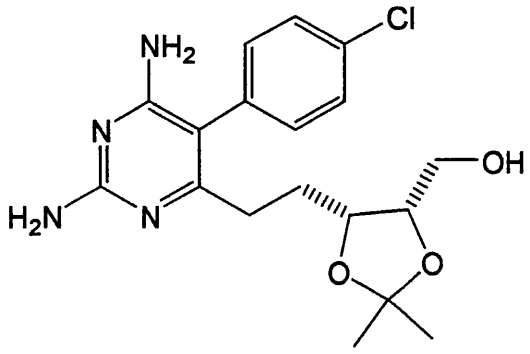
A solution of compound (136) (0.34 g, 1.1 mmol) in THF (5 ml) was treated with a solution of diazomethane (~0.08 g, 2.2 mmol) prepared from N-methyl-N-nitroso-p-toluenesulfonamide and KOH (using Minidiazald apparatus) in diethyl ether (5 ml) and the mixture was kept overnight at 10 °C. The excess of diazomethane was destroyed by dropwise addition of acetic acid (30% in THF), and the solvent was evaporated off to afford (140) (.0.33 g, 95%) as a pale yellow oil; NMR $\delta_H$ 1.40 (3H, s, Me), 1.51 (3H, s, Me), 1.87-2.01 (2H, m, C*H*$_2$CHO), 2.84-3.02 (2H, m, C*H*$_2$CH$_2$), 3.69 (2H, d, $J$ = 5.9 Hz, C*H*$_2$OH), 3.86 (3H, s, OCH$_3$), 4.22-4.27 (2H, m, 4,5-H$_2$), 7.24-7.42 (5H, m, Ph-H$_5$); MS *m/z* 318.1707 (M + H) ($C_{18}H_{24}NO_4$ requires 318.1705), 302 (M – $CH_3$), 277 (M - $C_2H_2N$), 258 (M – $C_2H_5NO$). A solution of sodium methoxide (0.17 g, 3.2 mmol) in 2-methoxyethanol (2 ml) was added to a solution of guanidine hydrochloride (0.30 g, 3.2 mmol) in 2-methoxyethanol (2 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (30) (0.5 g, 1.6 mmol) for 4 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (144) (0.23 g, 42%); $R_f$ = 0.29 as a buff solid: mp 72-75 °C; NMR ($D_2O$) $\delta_H$ 1.27 (3H, s, Me), 1.31 (3H, s, Me), 1.75-1.88 (2H, m, C*H*$_2$CHO), 2.32 (1H, ddd, $J$ = 15.9, 9.7, 6.0 Hz, C*H*CH$_2$), 2.49 (1H, ddd, $J$ = 15.9, 8.7, 7.4 Hz, C*H*CH$_2$), 3.53 (1H, dd, $J$ = 11.6, 5.9 Hz, C*H*OH), 3.65 (1H, dd, $J$ = 11.6,

5.9 Hz, C*H*OH), 4.05 (1H, q, $J$ = 12.2, 5.9 Hz, 4-H), 4.12 (1H, q, $J$ = 5.9 Hz, 5-H), 4.63 (2H, br, $NH_2$), 4.95 (2H, br, $NH_2$), 7.21(1H, d, $J$ = 7.9 Hz, Ph 2-H), 7.22 (1H, d, $J$ = 7.9 Hz, Ph 6-H), 7.36 (1H, t, $J$ = 7.9 Hz, Ph 4-H), 7.41(2H, t, $J$ = 7.9 Hz, Ph 3,5-$H_2$); NMR ($D_2O$) $\delta_C$ 23.01 (Me), 25.58 (Me), 25.70 ($CH_2$), 28.17 ($CH_2$), 58.60 (C-OH), 74.07 and 75.63 (4,5-C), 105.27 (Pyr 5-C), 106.26 (*C*$Me_2$), 125.50 (Ph CH), 126.82 (2 × Ph CH), 126.85 (2 × Ph CH), 127.94 (Ph 1-C), 158.71 (Pyr 2-C), 159.82 (Pyr 4-C), 162.92 (Pyr 6-C); MS *m/z* 345.1935 (M + H) ($C_{19}H_{24}N_4O_3$ requires 345.1926), 329 (M - $CH_3$), 200 (M – $C_7H_{12}O_3$).

**(4*R*,5*S*)-4-(4-(4-Chlorophenyl)-4-cyano-3-methoxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (141) and 2,4-diamino-5-(4-chlorophenyl)-6-(2-((4*R*,5*S*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine (145)**




**(141)**

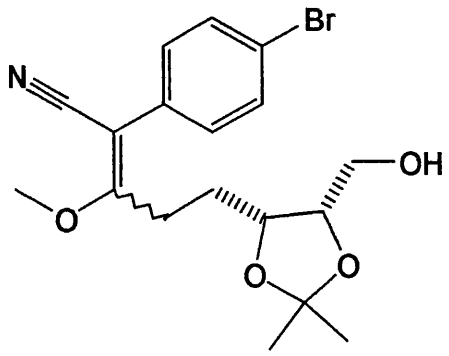



**(145)**

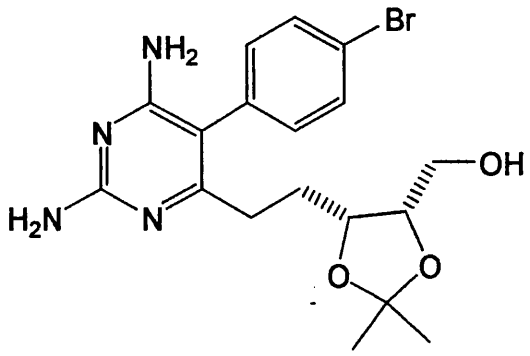
A solution of compound (137) (0.32 g, 1 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described above to afford (141) (0.3 g, 90%) as a pale yellow oil. A solution of sodium methoxide (0.12 g, 2.2 mmol) in 2-methoxyethanol (2 ml) was added to a solution of guanidine hydrochloride (0.21 g, 2.2 mmol) in 2-methoxyethanol (2 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (141) (0.3 g, 0.8 mmol) for 10 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (145) (50 mg, 12%); $R_f$ = 0.24 as a white solid: mp 94-95 °C; NMR $\delta_H$ 1.27 (3H, s, Me), 1.30 (3H, s, Me) 1.68-1.77 (1H, m, C*H*CHO), 1.79-1.88 (1H, m, C*H*CHO), 2.29 (1H,

ddd, $J$ = 13.4, 10.8, 5.1 Hz, C*H*$CH_2$), 2.46 (1H, ddd, $J$ = 13.4, 10.4, 5.9 Hz, C*H*$CH_2$), 3.56 (1H, dd, $J$ = 11.7, 6.1 Hz, C*H*OH), 3.66 (1H, dd, $J$ = 11.7, 6.1 Hz, C*H*OH), 4.02 (1H, dt, $J$ = 8.1, 5.8 Hz, 4-H), 4.13 (1H, q, $J$ = 5.8 Hz, 5-H), 5.04 (2H, br, $NH_2$), 5.76 (2H, br, $NH_2$), 7.16 (2H, d, $J$ = 7.0 Hz, Ph 3,5-$H_2$), 7.42 (2H, d, $J$ = 7.0 Hz, Ph 2,6-$H_2$); NMR $\delta_C$ 25.57 (Me), 28.04 (Me), 28.42 ($CH_2$), 30.46 ($CH_2$), 60.90 ($CH_2OH$), 76.77 and 77.09 (4,5-CH), 107.39 (*C*$Me_2$), 107.94 (Pyr 5-C), 129.71 (2 × Ph CH), 132.64 (Ph 1-C), 134.29 (Ph 4-C), 160.82 (Pyr 2-C), 162.48 (Pyr 4-C), 164.40 (Pyr 6-C); MS *m/z* 381.1506 (M + H) ($C_{18}H_{24}{}^{37}ClN_4O_3$ requires 381.1507), 379.1525 (M + H) ($C_{18}H_{24}{}^{35}ClN_4O_4$ requires 379.1536), 236/234 (M - $C_7H_{12}O_3$), 186 (M – $C_8H_{13}ClO_3$).

**(4*R*,5*S*)-4-(4-(4-Bromophenyl)-4-cyano-3-methoxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (142) and 2,4-diamino-5-(4-bromophenyl)-6-(2-((4*R*,5*S*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine (146)**




**(142)**

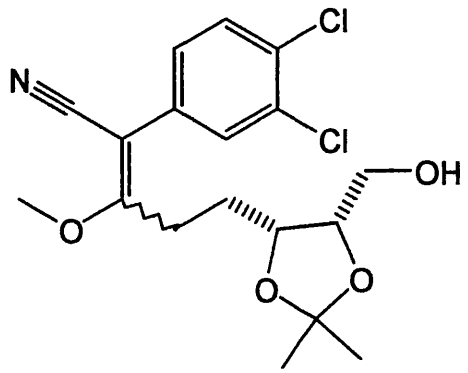



**(146)**

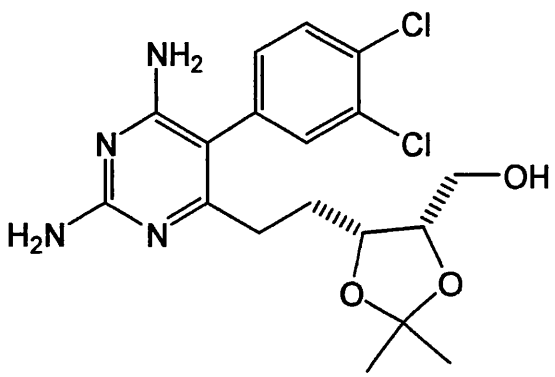
A solution of compound (138) (0.32 g, 0.84 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described above to afford (142) (0.30 g, 90%) as a pale yellow oil; IR $\nu_{max}$ 3435 (OH), 2243 (CN), 1592 (C=C) $cm^{-1}$; NMR $\delta_H$ 1.42 (3H, s, Me), 1.52 (3H, s, Me), 2.38-2.49 (2H, m, C*H*$_2$CHO), 2.74-2.80 (2H, m, C*H*$_2$CH$_2$), 3.33 (3H, s, $OCH_3$), 3.64 (1H, dd, $J$ = 11.1, 5.0 Hz, C*H*OH), 3.74 (1H, dd, $J$ = 11.1, 5.0 Hz, C*H*OH), 4.21-4.27 (2H, m, 4,5-$H_2$), 7.29 (2H, d, $J$ = 8.5 Hz, Ph 3,5-$H_2$), 7.50 (2H, d, $J$ = 8.5 Hz, Ph 2,6-$H_2$). A solution of sodium methoxide (0.10

g, 2 mmol) in 2-methoxyethanol (2 ml) was added to a solution of guanidine hydrochloride (0.18 g, 2 mmol) in 2-methoxyethanol (2 ml) mixed and stirred for 5 min at 30 °C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (142) (0.37 g, 1 mmol) for 10 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (146) (80 mg, 20%); $R_f$ = 0.11 as a white solid: mp 124-125 °C; NMR $\delta_H$ 1.27 (6H, s, $CMe_2$), 1.61-1.79 (2H, m, $CH_2CHO$), 2.26 (1H, ddd, $J$ = 13.0, 10.6, 5.8 Hz, $CHCH_2$), 2.46 (1H, ddd, $J$ = 13.0, 10.6, 5.8 Hz, $CHCH_2$), 3.50 (1H, dd, $J$ = 11.1, 5.9 Hz, $CHOH$), 3.54 (1H, dd, $J$ = 11.1, 5.9 Hz, $CHOH$), 4.01 (1H, ddd, $J$ = 9.9, 5.9, 3.9 Hz, 4-H), 4.08 (1H, q, $J$ = 5.9 Hz, 5-H), 7.21 (2H, d, $J$ = 7.7 Hz, Ph 3,5-$H_2$), 7.66 (2H, d, $J$ = 7.7 Hz, Ph 2,6-$H_2$); NMR ($CD_3OD$) $\delta_C$ 24.43 (Me), 27.11 (Me), 28.49 ($CH_2$), 30.66 ($CH_2$), 60.32 (C-OH), 76.63 and 77.89 (4,5-C), 107.20 ($CMe_2$), 107.82 (Pyr 5-C), 121.84 (Ph 4-C), 132.21 (2 × Ph CH), 132.57 (2 × Ph CH), 133.50 ( Ph 1-C), 160.82 (Pyr 2-C), 162.98 (Pyr 4-C), 163.71 (Pyr 6-C); MS *m/z* 425.1007 (M + H) ($C_{18}H_{24}{}^{81}BrN_4O_3$ requires 425.1011), 423.1019 (M + H) ($C_{18}H_{24}{}^{79}BrN_4O_4$ requires 423.1013), 280/278 (M - $C_7H_{12}O_3$), 186 (M – $C_8H_{13}BrO_3$).

**(4*R*,5*S*)-4-(4-(3,4-Dichlorophenyl)-4-cyano-3-methoxybut-3-enyl)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (143) and 2,4-diamino-5-(3,4-dichlorophenyl)-6-(2-((4*R*,5*S*)-5-hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)ethyl)pyrimidine (147)**




**(143)**




**(147)**

A solution of compound (139) (0.35 g, 0.94 mmol) in THF (5 ml) was methylated with diazomethane by the same procedure described above to afford (143) (0.33 g, 91%) as a pale yellow oil; IR $\nu_{max}$ 3467 (OH), 2210 (CN), 1597 (C=C) $cm^{-1}$; NMR $\delta_H$ 1.40 (3H, s, Me), 1.50 (3H, s, Me), 2.30-2.40 (2H, m, C*H*$_2$CHO), 2.72-2.80 (2H, m, C*H*$_2$CH$_2$), 3.37 (3H, s, OCH$_3$), 3.58-3.74 (2H, m, C*H*$_2$OH), 4.18-4.26 (2H, m, 4,5-H$_2$), 7.51 (1H, d, $J$ = 8.3 Hz, Ph 5-H), 7.84 (1H, dd, $J$ = 8.3, 2.0 Hz, Ph 6-H), 8.10 (1H, d, $J$ = 2.0 Hz, Ph 2-H). A solution of sodium methoxide (84 mg, 1.5 mmol) in 2-methoxyethanol (2 ml) was added to a solution of guanidine hydrochloride (143 mg, 1.5 mmol) in 2-methoxyethanol (2 ml) mixed and stirred for 5 min at 30°C and sodium chloride was removed. The alcoholic guanidine solution was boiled under reflux with compound (143) (0.3 g, 0.78 mmol) for 6 h. The cooled concentrated solution was purified by chromatography (chloroform/methanol, 9:1) to afford (147) (30 mg, 9%); $R_f$ = 0.30 as a white solid: mp 114-115 °C; NMR $\delta_H$ 1.29 (3H, s, Me), 1.33 (3H, s, Me), 1.74-1.81 (1H, m, C*H*CHO), 1.84-1.92 (1H, m, C*H*CHO), 2.26-2.42 (1H, m, C*H*CH$_2$), 2.44-2.52 (1H, m, C*H*CH$_2$), 3.58 (1H, dd, $J$ = 11.4, 5.9 Hz, C*H*OH), 3.67 (1H, dd, $J$ = 11.4, 5.9 Hz, C*H*OH), 4.02-4.07 (1H, m, 4-H), 4.15 (1H, q, $J$ = 5.6 Hz, 5-H), 4.95 (2H, br, NH$_2$), 5.53 (2H, br, NH$_2$), 7.08 (1H, dd, $J$ = 8.4, 1.7 Hz, Ph 6-H), 7.38 (1H, d, $J$ = 1.7 Hz, Ph 2-H), 7.52 (1H, d, $J$ = 8.4 Hz, Ph 5-H); NMR $\delta_C$ 25.53 (Me), 28.04 (Me), 28.12 (CH2), 30.35 (CH$_2$), 61.05 (C-OH), 65.83 and 70.51 (4,5-C), 106.62 (*C*Me$_2$), 108.04 (Pyr 5-C), 130.15 (2 × Ph CH), 131.41 (Ph CH), 131.45 (Ph C), 133.51 (Ph C), 134.40 (Ph C), 160.93 (Pyr 2-C), 162.22 (Pyr 4-C), 168.33 (Pyr 6-C); MS *m/z* 417.1091 (M + H) ($C_{18}H_{23}{}^{37}Cl_2N_4O_3$ requires 417.1088), 415.1103 (M + H) ($C_{18}H_{23}{}^{37}Cl\ {}^{35}ClN_4O_3$ requires 415.1117), 413.1129 (M + H) ($C_{18}H_{23}{}^{35}Cl_2N_4O_3$ requires 413.1147), 272/270/268 (M - $C_7H_{12}O_3$) 186 (M – $C_8H_{12}Cl_2O_3$).

## 5.2 Evaluation of Target Compounds

### Method

The gene modified yeast strains were kindly provided by Dr. Carol Hopkins Sibley, Department of Genome Sciences, University of Washington.

### Radial Spoke Assay

The TB-yeast, the human-yeast and the wild-type *Saccharomyces cerevisiae* were grown in media contain 10% yeast extract, 10% peptone and 10% dextrose. Sulfanilamide (1mM, 100 µl) was spread onto fresh plates and allowed to absorb into the medium overnight. A template streaked with the appropriate yeast that had grown for 3 days was sequentially replica plated onto these plates, and tested compound (10 µl, 10 mM in DMSO) was placed in the center of the plate. Assay plates were incubated for 3 days at 30 °C to allow growth of the yeast before the inhibition zone was measured. The inhibition zones that were at least 4.0 mm more than that from the control plate with DMSO alone were scored as inhibited by the drug on the plate. The inhibition zones were calculated from the mean of three different experiments and the standard deviations were calculated.

# REFERENCES


1) Skerman, V. D. B.; McGowan, V.; Sneath, P. H. A. *Int. J. Syst. Bacteriol.* **1980**, *30*, 225-420.
2) Shinnick, T. M.; Good, R. C. *Eur. J. Clin. Microbiol. Infect. Dis.* **1994**, *13*, 388-391.
3) Levy-Frebault, V.; Portaels, F. *Int. J. Syst. Bacteriol.* **1992**, *42*, 315-323.
4) Rogall, T. F.; Flohr, T.; Bottger, E. C. *J. Gen. Microbiol.* **1990**, *136*, 1915-1920.
5) Paul, T. R.; Beveridge, T. J. *J. Bacteriol.* **1992**, *174*, 6508-6517.
6) Wayne, L. G.; Sramek, H. A. *Clin. Microbiol. Rev.* **1992**, *5*, 1-25.
7) Eisenstadt, J.; Hall, G. S. *Clin. Dermatol.* **1995**, *13*, 197-206.
8) Griffiths, P. A.; Babb, J. R.; Fraise, A. P. *J. Hosp. Infect.* **1999**, *41*, 111-121.
9) Bishop, B. J.; Newmann, G. *Tubercle* **1970**, *51*, 196-206.
10) Kim, J. Y.; Shakow, A.; Mate, K.; Vanderwaker, C.; Gupta, R.; Farmer, P. *Soc. Sci. Med.* **2005**, *61*, 847-859.
11) Srivastava, R.; Kumar, D.; Subramaniam, P.; Srivastava, B. S. *Biochem. Biophys. Res. Comm.* **1997**, *235*, 602-605.
12) Grange, J. M. In: Mycobacteria and Human Disease, 2nd ed., Arnold, London, Sydney **1996**, 41-62.
13) Crofton, J. *J. Pharm. Pharmacol.* **1997**, 3-6.
14) Malaviya, A. N.; Kotwal, P. P. *Best Pract. Res. Clin. Rheumatol.* **2003**, *17*, 319-343.
15) World Health Organization, Tuberculosis fact sheet (No. 104) 200; (www.who.int/factsheets/), 12 April 2005.
16) Kazimerczuk, Z.; Andrzejewska, M.; Kaustova, J.; Klimesova, V. *Eur. J. Med. Chem.* **2005**, *40*, 203-208.
17) Dye, C.; Schelle, S.; Dolin, P.; Pathania, V.; Raviglione, M. C. *JAMA.* **1999**, *282*, 677-686.
18) Grange, J. M. In Mycobacteria and Human Disease, 2nd ed., Arnold, London, Sydney **1996**, 159-185.
19) Mackaness, G. *Am. Rev. Resp. Dis.* **1968**, *97*, 337-344.

20) Curvo-Semedo, L.; Teixeira, L.; Caseiro-Alves, F. *Eur. J. Radiol.* **2005** (In Press).

21) Cole, S. T.; Brosch, R.; Parkhill, J. Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; Mclean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborn, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G. *Nature* **1998**, *393*, 537-544.

22) Grange, J. M. In: Mycobacteria and Human Disease, 2nd ed., Arnold, London, Sydney **1996**, 83-102.

23) Goodwin, R. A.; Desprez, R. M. *Chest* **1983**, *83*, 801-805.

24) Slavin, R. E.; Walsh, T. J.; Pollack, A. D. *Medicine* (Baltimore) **1980**, *59*, 352-366.

25) Yoon, H. Y.; Song, Y. G.; Park, W. I.; Choi, J. P.; Chang, K. H.; Kim, J. M. *Yonsei Med. J.* **2004**, *45*, 453-461.

26) Shin, S.; Furin, J.; Bayona, J.; Mate, K.; Kim, J. Y.; Farmer, P. *Soc. Sci. Med.* **2004**, *59*, 1529-1539

27) Shaw, R. J. *J. Pharm. Pharmacol.* **1997**, 17-20.

28) Dutt, A. K.; Stead, W. *Disease-a-Month* **1997**, *43*, 247-274.

29) Mitchison, D. A. *J. Pharm. Pharmacol.* **1997**, 31-36.

30) Zhang, Y.; Amzel, L. M. *Current Drug Targets* **2002**, *3*, 131-154.

31) Zhang, Y.; Heym, B. Allen, B.; Young, D.; Cole, S. *Nature* **1992**, *358*, 591-593.

32) Smith, C. V.; Sharma, V.; Sacchettini, J. C. *Tuberculosis* **2004**, *84*, 45-55.

33) Cynamon, M. H.; Klemens, S. P.; Chou, T.-S.; Gimi, R. H.; Welch, J. T. *J. Med. Chem.* **1992**, *35*, 1212-1215.

34) Zhang, Y.; Scorpio, A.; Nikaido, H.; Sun, Z. *J. Bacteriol.* **1999**, *181*, 2044-2049.

35) Gasymov, O. K.; Abduragimov, A. R.; Gasimov, E. O.; Yusifov, T. N.; Dooley, A. N.; Glasgow, B. *J. Biochem. Biophy. Acta* **2004**, *1688*, 102-111.

36) Mikusova, K.; Slayden, R. A.; Besra, G. S.; Brennan, P. J. *Antimicrob. Agents Chemother.* **1995**, *39*, 2484-2489.

37) Chenevier, P.; Massias, L.; Gueylard, D.; Farinotti, R. *J. Chromatog. B* **1998**, *708*, 310-315.

38) Takayama, K.; Kilburn, J. O. *Antimicrob. Agents Chemother.* **1989**, *33*, 1493-1499.

39) Wolucka, B. A.; McNeil, M. R.; de Hoffmann, E.; Chojnacki, T.; Brennan, P. J. *J. Biol. Chem.* **1994**, *269*, 23328-23335.

40) DeCock, K. M.; Chaisson, R. E. *Int. J. Tubercul. Lung Dis.* **1999**, *3*, 457-465.

41) Oslen, A. W.; Andersen, P. *Immunol. Lett.* **2003**, *85*, 207-211.

42) Migliori, G. B.; Raviglione, M. C.; Schaberg, T.; Davies, P. D. O.; Zellweger, J. P.; Grzemska, M.; Mihaescu, T.; Clancy, L.; Casali, L. *Eur. Resp. J.* **1999**, *14*, 978-992.

43) Wong, K. F.; Watney, J. B.; Hammes Schiffer, S. *J. Phys. Chem. B* **2004**, *108*, 12231-12241.

44) Johnson, J. M.; Melering, E. M.; Wright, J. E.; Pardo, J.; Rosowsky, A.; Wagner, G. *Biochemistry* **1997**, *36*, 4399-4411.

45) Mathews, C. K.; Holde, K. E. V. In: Biochemistry, 2nd ed., The Benjamin/Cummings Pub. Co., Inc., California, New York **1996**, 724-733.

46) Blaney, J. M.; Hansch, C.; Silipo, C.; Vittoria, A. *Chem. Rev.* **1984**, *84*, 333-407.

47) Li, D.; Levy, S. G.; Lebetkin, E. F. D.; Wall, M. J.; Howell, E. E.; London, R. E. *Biochemistry* **2001**, *40*, 4242-4252.

48) Cangjee, A.; Yu, J.; McGuire, J. J.; Cody, V.; Galitsky, N.; Kisliuk, R. L.; Queener, S. F. *J. Med. Chem.* **2000**, *43*, 3837-3851.

49) Baker, B. R.; Huang, P. C.; Meyer, R. B. *J. Med. Chem* **1968**, *11*, 475-482.

50) Griffin, R. J.; Meek, M. A.; Schwalbe, C. H.; Stevens, M. F. G. *J. Med. Chem.* **1989**, *32*, 2468-2474.

51) Cody, V.; Gulitsky, N.; Rak, D.; Luft, D. R.; Pangborn, W.; Queener, S. F. *Biochemistry* **1999**, *38*, 4303-4312.

52) Debnath, A. K. *J. Med. Chem.* **2002**, *45*, 41-53.

53) Kaneshiro, E. S. *Int. J. Parasitol.* **1998**, *28*, 65-84

54) Cody, V.; Chan, D.; Galitsky, N.; Rak, D.; Luft, J. R.; Pangborn, W.; Queener, S. F.; Laughton, C. A.; Stevens, M. F. G. *Biochemistry* **2000**, *39*, 3556-3564.

55) Franklin, T. J.; Snow, G. A. In: Biochemistry and Molecular Biology of Antimicrobial Drug Action, 5th ed., Kluwer Academic Publisher, Dordrecht, Boston, London **1998**, 63-66.

56) Brown, C. W.; Liu, S.; Klucik, J.; Berlin, D.; Brennan, P. J.; Kaur, D.; Benbrook, D. M. *J. Med. Chem.* **2004**, *47*, 1008-1017.

57) Kharkar, P. S.; Kulkarni, V. M. *Org. Biomol. Chem.* **2003**, *1*, 1315-1322.

58) McGourt, M.; Cody, V. *J. Am. Chem. Soc.* **1991**, *113*, 6634-6639.

59) Baker, B. R. *J. Pharm. Sci.* **1964**, *53*, 347-364.

60) Lemck, L.; Christensen, I. T.; Jorgensen, F. S. *Bioorg. Med. Chem.* **1999**, *7*, 1003-1011.

61) Magg, H. M.; Locher, R.; Daly, J. J.; Kopis, I. *Helv. Chim. Acta* **1986**, *69*, 887-896.

62) Chunduru, S. K.; Cody, V.; Luft, J. R.; Pangborn, W.; Appleman, J. R.; Blakley, R. L. *J. Biol. Chem.* **1994**, *269*, 9547-9555.

63) Otzen, T.; Wempe, E. G.; Kunz, B.; Bartels, R.; Lehwark-Yvetot, G.; Hansel, W.; Schaper, K.; Seydel, J. K. *J. Med. Chem.* **2004**, *47*, 240-253.

64) Pathak, A. K.; Pathak, V.; Seitz, L. E.; Suling, W. J.; Reynolds, R. C. *J. Med. Chem.* **2004**, *47*, 273-276.

65) Suling, W. J.; Seitz, L. E.; Pathak, V.; Westbrook, L.; Barrow, E. W.; Ginkel, S. Z.; Reynolds, R. C.; Piper, J. R.; Barrow, W. W. *Antimicrob. Agents Chemother.* **2000**, *44*, 2784-2793.

66) Seydel, J. K. *J. Chemother.* **1993**, *5*, 422-429.

67) Stevens, M. F. G.; Phillips, K. S.; Rathbone, D. L.; O'Shea, D. M.; Queener, S. F.; Schwalbe, C. H.; Lambert, P. A. *J. Med. Chem.* **1997**, *40*, 1886-1893.

68) Tarnchompoo, B.; Sirichaiwat, C.; Phupong, W.; Intaraudom, C.; Sirawaraporn, W.; Kamchonwongpaisan, S.; Vanichtanankul, J.; Thebtaranonth, Y.; Yuthavong, Y. *J. Med. Chem.* **2002**, *45*, 1244-1252.

69) Ganjee, A.; Adair, O.; Queener, S. *Bioorg. Med. Chem.* **2001**, *9*, 2929-2935.

70) Graffner-Nordberg, M.; Kolmodin, K.; Aqvist, J.; Queener, S. F.; Hallberg, A. *J. Med. Chem.* **2001**, *44*, 2391-2402.

71) Robson, C.; Meek, M. A.; Gruwaldt, J. D.; Lambert, P. A.; Queener, S. F.; Schmidt, D.; Griffin, R. J. *J. Med. Chem.* **1997**, *40*, 3040-3048.

72) Ginkel, S. Z.; Dooley, T. P.; Suling, W. J.; Barrow, W. W. *FEMS Microbiol. Lett.* **1997**, *156*, 69-78.

73) Kuyper, L. F.; Baccanari, D. P.; Jones, M. L.; Hunter, R. N.; Tanik, R. L.; Joyner, S. S.; Boytes, C. M.; Rudolph, S. K.; Knick, V.; Wilson, H. R.; Caddell, J. M.; Friedman, H. S.; Comley, J. C. W; Stales, J. N. *J. Med. Chem.* **1996**, *39*, 892-903.

74) DeGraw, J. I.; Brown, V. H.; Colwell, W. T. *J. Med. Chem.* **1974**, *17*, 762-764.

75) Whitlow, M.; Howard, A. J.; Stewart, D.; Hardman, K. D. *J. Biol. Chem.* **1997**, *272*, 30289-30298.

76) Suling, W. J.; Reynolds, R. C.; Barrow, E. W.; Wilson, L. N.; Piper, J. R.; Barrow, W. W. *J. Antimicrob. Chemother.* **1998**, *42*, 811-815.

77) Sirichaiwat, C.; Intaraudom, C.; Kamchonwongpaisan, S.; Vanichtanankul, J.; Thebtaranonth, Y.; Yuthavong, Y. *J. Med. Chem.* **2004**, *47*, 345-354.

78) Gangiee, A.; Lin, X.; Queener, S. F. *J. Heterocycl. Chem.* **2003**, *40*, 507-512.

79) Nelson, R. G.; Rosowsky, A. *Antimicrob. Agents Chemother.* **2001**, *45*, 3293-3303.

80) Bonde, C. G.; Gaikwad, N. J. *Bioorg. Med. Chem.* **2004**, *12*, 2151-2161.

81) Richardson, M. L.; Croughton, K. A.; Mathews, C. S.; Stevens, M. F. G. *J. Med. Chem.* **2004**, *47*, 4105-4108.

82) Denny, B. J.; Ringan, N. S.; Schwalbe, Lambert, P. A.; Meek, M. A.; Griffin, R. J.; Stevens, M. F. G. *J. Med. Chem.* **1992**, *35*, 2315-2320.

83) Li, R.; Sirawaraporn, R.; Chitnumsub, P.; Sirawaraporn, W.; Wooden, J.; Athappilly, F.; Turley, S.; Hol, W. G. J. *J. Mol. Biol.* **2000**, *295*, 307-323.

84) Cody, V.; Galitsky, N.; Luft, J. R.; Pangborn, W.; Blakley, R. L.; Gangjee, A. *Anti-Cancer Drug Design*, **1998**, *13*, 307-315.

85) Gangjee, A.; Guo, X.; Queener, S.F.; Cody, V.; Galitskey, N.; Luft, J. R.; Pangborn, W. J.; Pangborn, W. *J. Med.Chem.* **1998**, *41*, 1263-1271.

86) Forsch, R. A.; Queener, S. F.; Rosowsky, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1811-1815.

87) Shoen, C. M.; Choromanska, O.; Reynolds, R. C.; Piper, J. R.; Johnson, C. A.; Cynamon, M. H. *Antimicrob. Agents Chemother.* **1998**, *42*, 3315-3316.

88) Gorse, A. D.; Gready, J. E. *Protein Eng.* **1997**, *10*, 23-30.

89) Cody, V.; Luft, J. R.; Ciszak, E.; Kalman, T. I.; Freisheim, J. H. *Anti-Cancer Drug Design* **1992**, *7*, 483-491.

90) Opravil, M.; Pechere, M.; Lazzarin, A.; Heald, A.; Ruttimanns, S.; Iten, A.; Furrer, H.; Oertle, D.; Praz, G.; Vuitton, D. A.; Hirschel, B.; Luthy, R. *Clin. Infect. Dis.* **1995**, *20*, 244-249.

91) Billington, D. C.; Coleman, M. D.; Ibiabuo, J.; Lambert, P. A.; Rathbone, D. L.; Tims, K. J. *Drug Design and Discovery* **1998**, *15*, 269-275.

92) Rosowsky, A.; Fu, H.; Chan, D. C. M.; Queener, S. F. *J. Med. Chem.* **2004**, *47*, 2475-2485.

93) Mathews, D. A.; Alden, R. A.; Bolin, J. T.; Freer, S. T.; Hamlin, R.; Xunong, N.; Kraut, J. *Science* **1977**, *197*, 452-455.

94) Gargaro, A. R.; Soterious, A.; Frenkiel, T. A.; Bauer, C. J.; Birdsall, B.; Polshakov, V. I.; Barsukov, I. L.; Roberts, G. C. K.; Feeney, J. *J. Mol. Biol.* **1998**, *277*, 119-134.

95) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205-1212.

96) Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J.; Norrington, F. E. A.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1982**, *25*, 1120-1123.

97) Selassie, C. D.; Fang, Z.; Li, R.; Hansch, C.; Debnath, G.; Klein, T. E.; Langridge, R.; Kaufman, B. T. *J. Med. Chem.* **1989**, *32*, 1895-1905.

98) Kim, K. H.; Hansch, C.; Fukunaga, J. Y.; Steller, E. E.; Jow, P. Y. C.; Craig, P. N.; Page, J. *J. Med. Chem.* **1979**, *22*, 366-391.

99) Silverman, R. B. In: the Organic Chemistry of Drug Design and Drug Action, 2nd ed., Elsevier Academic Press, Amsterdam, Boston **2004**, 75-89.

100) http:// www.rcb.org/pdb, 15 June 2005.

101) Ferrin, T. E.; Huang, C. C.; Jarvis, L. E. *J. Mol. Graphics* **1988**, *6*, 13-27.

102) Huang, C. C.; Pettersen, E. F.; Klein, T.E.; Ferrin, T. E.; Langridge, R. *J. Mol. Graphics* **1991**, *9*, 230-239.

103) Cody, V.; Galitsky, N.; Luft, J. R.; Pangborn, W.; Rosowsky, A.; Blakley, R. L. *Biochemistry* **1997**, *36*, 13897-13903.

104) Davies, J. F. D.; Delcamp, T. J.; Prendergast, N. J.; Ashford, V. A.; Frisheim, J. H.; Kraut, J. *Biochemistry* **1990**, *29*, 9467-9479.

105) Shah, L. M.; Meyer, S. C.; Cynamon, M. H. *Antimicrob. Agents Chemother.* **1996**, *40*, 2426-2427.

106) Czaplinski, K. H.; Hansel, W.; Wiese, M.; Seydel, J. K. *Eur. J. Med. Chem.* **1995**, *30*, 779-787.

107) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G; Taylor, R. *J. Chem. Soc. Perkin Trans. 2* **1987**, SI-S19.

108) Brown, D. J.; Mason, S. F. In: Heterocylic Compounds, Pyrimidine, Interscience Publishers, New York, London **1962**, 306-313.

109) Russell, P. B.; Hitchings, G. H.; Chase, B. H.; Walker, J. *J. Am. Chem. Soc.* **1952**, *74*, 5403-5405.

110) Russell, P. B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1951**, *73*, 3763-3770.

111) Falcon, E. A.; DuBreuil, S.; Hitchings, G. *J. Am. Chem. Soc.* **1951**, *73*, 3758-3762.

112) Falcon, E. A.; Russell, P.B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1951**, *73*, 3753-3758.

113) Elion, G. B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1947**, *69*, 2138-2139.

114) Russell, P. B.; Elion, E. A.; Hitchings, F.; Hitchings, G. H. *J. Am. Chem. Soc.* **1949**, *71*, 2279-2282.

115) Rupe, H.; Huber, A. *Helv. Chim. Acta* **1927**, *10*, 846-850.

116) Cooke, G.; Augier de Cremiers, A.; Rotello, V. M.; Tarbit, B.; Vanderstraeten, P. E. *Tetrahedron* **2001**, *57*, 2787-2789.

117) Reeve, W.; Erikson, C. M.; Aluotto, P. F. *Can. J. Chem.* **1979**, *57*, 2747-2754.

118) Pearson, R. G.; Dillon, R. L. *J. Am. Chem. Soc.* **1953**, *75*, 2439-2443.

119) Quindon, Y.; Morton, H. E.; Yoakm, C. *Tetrahedron Lett.* **1983**, *24*, 3969-3972.

120) Coutts, L. D.; Cywin, C. L.; Kallmerten, J. *Synlett* **1993**, 696-698.

121) Tanabe, M.; Bigley, B. *J. Am. Chem. Soc.* **1961**, *83*, 756-757.

122) Kocienski, P. J. In: Protecting Groups, 3rd ed., Georg Thieme, Stuttgart, New York **2004**, 188-350.

123) Speziale, J.; Ratts, K. W. *J. Am. Chem. Soc.* **1963**, *85*, 2790-2795.

124) Katritzky, A. R.; Brycki, B. E. *Chem. Soc. Rev.* **1990**, *19*, 83-105.

125) Zoltewicz, J. A. *Top. Curr. Chem.* **1975**, *59*, 33-64.

126) Jia, Z. S.; Qi, C. Z.; Wu, L. M.; Yang, D. L.; Liu, Y. C. *Res. Chem. Intermed.* **1996**, *22*, 31-41.

127) Michael, L. C.; Davidsen, S. K.; Heyman, H. R.; Garland, R. B.; Sheppard, G. S.; Florjancic, A. S.; Xu, L.; Carrera, G. M.; Steinman, D. H.; Trautmann, J.

A.; Albert, D. H.; Magoc, T. J.; Tapang, P.; Rhein, D. A.; Conway, R. G.; Luo, G.; Denissen, J. F.; Marsh, K. C.; Morgan, D. W.; Summers, J. B. *J. Med. Chem.* **1998**, *41*, 74-95.

128) Beugelmans, R.; Singh, G. P.; Bois-Choussy, M.; Chastanet, J.; Zhu, J. *J. Org. Chem.* **1994**, *59*, 5535-5542.

129) Boga, C.; Forlani, L.; Guardia, P. *Gazz. Chim. Ital.* **1997**, *127*, 259-262.

130) Broxton, T. J.; Deady, L. W. *J. Org. Chem.* **1974**, *39*, 2767-2769.

131) Bellucci, C.; Gualtieri, F.; Scapecchi, S.; Teodori, E. *Farmaco* **1989**, *44*, 1167-1191.

132) Bliss, E. A.; Griffin, R. J.; Stevens, M. F. G. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2217-2228.

133) Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *J. Org. Chem.* **1990**, *55*, 4683-4687.

134) Baxter, J. N.; Perlin, A. S. *Can. J. Chem.* **1960**, *38*, 2217-2225.

135) Lerner, L. M. *Carbohyd. Res.* **1969**, *9*, 1-4.

136) Thompson, D. K.; Hubert, C. N.; Wightman, R. H. *Tetrahedron* **1993**, *49*, 3827-3840.

137) Pigman, W. In: The Carbohydrates, Chemistry, Biochemistry, Physiology, Academic Press Inc. Publishers, New York **1957**, 345-350.

138) Kadota, I.; Kadowaki, C.; Yoshida, N.; Yamamoto, Y. *Tetrahedron Lett.* **1998**, *39*, 6369-6372.

139) Buchanan, J. G.; Edgar, A. R.; Hewitt, B. D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2371-2376.

140) Murrer, B. A.; Brown, J. M.; Chaloner, P. A.; Nicholson, P. N.; Paker, D. *Synthesis* **1979**, 350-352.

141) Batty, D.; Crich, D. *Tetrahedron Lett.* **1992**, *33*, 875-878.

142) Batty, D.; Crich, D. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3193-3204.

143) Piancatelli, G.; Scettri, A.; D'Auria, M. *Synthesis* **1982**, 245-258.

144) Hunsen, M. *Tetrahedron Lett.* **2005**, *46*, 1651-1653.

145) Mukaiyama, T.; Suzuki, K.; Yamada, T.; Tabusa, F. *Tetrahedron* **1990**, *46*, 265-276.

146) Harris, J. M.; Liu, Y.; Chai, S.; Andrews, M. D.; Vederas, J. C. *J. Org. Chem.* **1998**, *63*, 2407-2409.

147) Liu, Y.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 7856-7859.

148) Maiti, G.; Roy, S. C. *Tetrahedron Lett.* **1997**, *38*, 495-498.

149) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190-6191.

150) Karimi, B.; Golshani, B. *J. Org. Chem.* **2000**, *65*, 7228-7230.

151) Kamiotis, D.; Pananookooln, S.; Zaw, K.; Dieter, J. P.; Le Berton, G. C.; Venton, D. L. *Eur. J. Med. Chem.* **1995**, *30*, 321-326.

152) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799-3807.

153) Khalafi-Nezhad, A.; Almaddari, R. F.; Zekri, N. *Tetrahedron* **2000**, *56*, 7503-7506.

154) Clader, J. W.; Berger, J. G.; Burrier, R. E.; Davis, H. R.; Domalski, M.; Dugar, S.; Kogan, T. P.; Salisbury, B.; Vaccaro, W. *J. Med. Chem.* **1995**, *38*, 1600-1607.

155) Landau, E. F.; Leonard, F.; Carroll, R. H.; Sporri, P. E. *J. Am. Chem. Soc.* **1945**, *67*, 564-565.

156) Das, B. P.; Boykin, D. W. *J. Med. Chem.* **1974**, *17*, 372-374.

157) Cook, C. E.; Corley, R. C.; Wall, M. E. *J. Org. Chem.* **1965**, *30*, 4114-4120.

158) Eby, C. J.; Hauser, C. R. *J. Am. Chem. Soc.* **1957**, *79*, 723-725.

159) Levine, R.; Hauser, C. R. *J. Am. Chem. Soc.* **1946**, *68*, 760-761.

160) Kayaleh, N. E.; Gupta, R. C.; Johnson, F. *J. Org. Chem.* **2000**, *65*, 4515-4522.

161) Bordwell, F. G.; Bares, J. E.; Bartmess, J. E.; McCollum, G. J.; Van Der Puy, M.; Vanier, N. R.; Mathews, W. S. *J. Org. Chem.* **1977**, *42*, 321-325.

162) Watt, D. S. *Tetrahedron Lett.* **1974**, *9*, 707-710.

163) Arseniyadis, S.; Kyler, K. S; Watt, D. S. *Org. React.* **1984**, *31*, 31-40.

164) Zarrinmayeh, H.; Bleakman, D.; Gutles, M. R.; Yu, H.; Zimmerman, D. M.; Ornstein, P. L.; Mckennon, T.; Arnold, M. B.; Wheeler, W. J.; Skolnick, P. *J. Med. Chem.* **2001**, *44*, 302-304.

165) Williams, D. H.; Fleming, I. In: Spectroscopic Methods in Organic Chemistry, 5th ed., McGraw-Hill, London **1995**, 120-161.

166) Hart, H. *Chem. Rev.* **1979**, *79*, 515-528.

167) Chase, B. H.; Walker, J. *J. Chem. Soc.* **1953**, *54*, 3518-3525.

168) Cravero, M.; Gonzalez-Sierra, M.; Oliver, A. C. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1067-1071.

169) Rappoport, Z.; Biali, S. E. *Acc. Chem. Res.* **1988**, *21*, 442-449.

170) Kaftory, M.; Nugiel, Biali, S. E.; Rappoport, Z. *J. Am. Chem. Soc.* **1989**, *111*, 8181-8191.

171) Ngan, F.; Toofan, M. *J. Chromatogr. Sci.* **1991**, *29*, 8-10.

172) Arndt, F.; Blatt, A. H. *Org. Synth.* **1950**, *2*, 165-167.

173) McKay, A. F. *J. Am. Chem. Soc.* **1948**, *70*, 1974-1975.

174) DeBoer, T. J.; Backer, H. J.; Rabjohn, N. *Org. Synth.* **1963**, *4*, 250-253.

175) Lombardo, L. *Tetrahedron Lett.* **1984**, *25*, 227-228.

176) Kadota, I.; Kawada, M.; Gevorgyan, V.; Yamamoto, Y. *J. Org. Chem.* **1997**, *62*, 7439-7446.

177) Suzuki, T.; Matsumura, R.; Oku, K.; Taguchi, K.; Hagiwara, H.; Hoshi, T.; Ando, M. *Tetrahedron Lett.* **2001**, *42*, 65-67.

178) Nicolaou, K. C.; Reddy, K. R.; Skokotas, G.; Sato, F.; Xiao, X.-Y.; Hwang, C.-K. *J. Am. Chem. Soc.* **1993**, *115*, 3558-3575.

179) Nicolaou, K. C.; Hummel, C. W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126-3128.

180) Uwai, K.; Oshima, Y. *Tetrahedron* **1999**, *55*, 9469-9480.

181) Wyss, P. C.; Gerber, P.; Hartman, P. G.; Hubschwerlen, C.; Locher, H.; Marty, H.; Stahl, M. *J. Med. Chem.* **2003**, *46*, 2304-2312.

182) Wang, X. J.; Hart, S. A.; Xu, B.; Mason, M. D.; Goodell, J. R.; Etzkorn, F. A. *J. Org. Chem.* **2003**, *68*, 2343-2349.

183) Park, M. H.; Takeda, R.; Nakanishi, K. *Tetrahedron Lett.* **1987**, *28*, 3823-3824.

184) Threadgill, M. D.; Griffin, R. J.; Stevens, M. F. G.; Wong, S. K. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2229-2234.

185) Gerum, A. B.; Ulmer, J. E.; Jacobus, D. P.; Jensen, N. P.; Sherman, D. R.; Sibly, C. H. *Antimicrob. Agents Chemother.* **2002**, *46*, 3362-3369.

186) Iseman, M. D. In: A Clinician's Guide to Tuberculosis, Lippincott Williams and Wilkins, New York, London **2000**, 30-31.

187) Prescott, L. M.; Harley, J.; Klein, D. A. In: Microbiology, 4th, ed., McGraw-Hill, Boston **1999**, 310-315.

188) Thomas, G. In: Medicinal Chemistry an introduction, John Wiley and Sons, Ltd, Chichester, New York **2000**, 420-428.

189) Brophy, V. H.; Vasquez, J.; Nelson, R. G.; Forney, J. R.; Rosowsky, A.; Sibly, C. H. *Antimicrob. Agents Chemother.* **2000**, *44*, 1019-1028.

190) Quan, S.; Venter, H.; Dabbs, E. R. *Antimicrob. Agents Chemother.* **1997**, *41*, 2456-2460.

191) Mdluli, K.; Sherman, D. R.; Hickey, M. J.; Kreiswirth, B. N.; Morris, S.; Stover, C. K. Barry, C. E. *J. Infect. Dis.* **1996**, *174*, 1085-1090.

192) Brown, T. A. In: Gene Cloning an introduction, Stanley Thornes (Publishers) Ltd., Manchester **1998**, 133-142.

193) Mumberg, D.; Muller, R.; Funk, M. *Gene* **1995**, *156*, 119-122.

194) Gonzalez, A. H.; Berlin, O. G.; Bruckner, D. A. *J. Antimicrob. Chemother.* **1989**, *24*, 19-22.

195) Sibly, C. H.; Brophy, V. H.; Cheesman, S.; Hamihon, K. L.; Hankins, E. G.; Wooden, J. M.; Kilbey, B. *Methods* **1997**, *13*, 190-207.

196) Carmack, M.; Kelley, C. J. *J. Org. Chem.* **1967**, *33*, 2171-2173.

197) Hungerbuhler, E.; Seebach, D. *Helv. Chim. Acta* **1981**, *64*, 687-702.